SANITATION GAPS AMONG LOW-INCOME URBAN POPULATIONS

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SANITATION GAPS AMONG LOW-INCOME URBAN POPULATIONS

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All living things eat, so

everyone poops.

-Taro Gomi

To Anna. Thank you for being my constant reminder that there are more important things in life than a PhD.

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microliter	µL
ninety-five percent confidence interval	95% CI
American Community Survey	ACS
American Housing Survey	AHS
adjusted prevalence ratio	aPR
adjusted risk ratio	aRR
Clostridium difficile	*C. difficile*
community based organization	CBO
colony-forming unit	CFU
centimeter	cm
Mozambican national committee for bio-ethics and health	CNBS
quantification cycle	Cq
communal sanitation block	CSB
Maputo City Water and Sanitation Department	DAS
difference-in-difference	DID
deoxyribonucleic acid	DNA
Escherichia coli	*E. coli*
Enteroaggregative E. coli	EAEC
Enteroinvasive E. coli	EIES
Environmental Protection Agency	EPA
Enteropathogenic E. coli	EPEC
Enterotoxigenic E. coli	ETEC

fecal indicator bacteria	FIB
fecal sludge management	FSM
gram	g
generalized estimating equations	GEE
generalized linear model	GLM
generalized linear mixed-effect model	GLMM
global position system	GPS
Housing and Urban Development	HUD
integrated public use microdata set	IPUMS
Japanese Social Development Fund	JSDF
low- and middle-income countries	LMIC
Localized Sanitation Status Index	LSSI
Maputo Sanitation	MapSan
milligram	mg
milliliter	mL
Escherichia virus MS2	MS2
microbial source tracking	MST
number	n
non-governmental organization	NGO
degrees Celsius	^{o}C
degrees Fahrenheit	^{o}F
open defecation	OD
polymerase chain reaction	PCR
point-in-time	PIT

public use microdata set	PUMS
quantitative polymerase chain reaction	qPCR
respondent one	R1
respondent two	R2
randomized controlled trial	RCT
ribonucleic acid	RNA
risk ratio	RR
reverse transcriptase polymerase chain reaction	RT-PCR
Severe acute respiratory syndrome coronavirus 2	SARS-Cov-2
standard deviation	SD
Sustainable Development Goals	SDG
safe drinking water information system	SDWIS
shared latrine	SL
sub-Saharan Africa	SSA
shiga toxin-producing *E. coli*	STEC
soil-transmitted helminth	STH
taqMan array card	TAC
United Kingdom	UK
United Nations	UN
United States	US
Urban Sanitation Status Index	USSI
Water, sanitation, and hygiene	WASH
WASH Benefits Study	WASH-B

World Health Organization / United Nations Children's Fund Joint Monitoring Programme	WHO/UNICEF JMP
Water and Sanitation Programme	WSP
Water and Sanitation for the Urban Poor	WSUP
wastewater treatment plant	WWTP

SUMMARY

Most of the 2 billion people who still lack access to basic sanitation services (e.g. improved sanitation infrastructure) live in low- and middle-income countries or in underserved communities in economically rich countries. In such settings, progress towards universal safely managed sanitation may be evaluated in terms of access, function, and health impact. In the US, sustained access to flush toilets is a barrier for people experiencing homelessness. On the other hand, in low- and middle-income countries the function and health impact of sanitation services are important considerations alongside access. In Sub-Saharan Africa most urban residents (56%) do not have access to basic sanitation and in many cities the majority of fecal wastes are not yet safely managed.[1,2] Here we present the results of a desk-based study that estimated the number of people in the urban US without consistent access to flush toilets, a study in Atlanta, GA, USA that investigated access to sanitation services, and studies in Maputo, Mozambique that investigated the impact of an on-site sanitation intervention on pit-emptying and environmental fecal contamination, the potential use of fecal sludges from on-site sanitation systems for pathogen surveillance, and infection risks to children posed by *Shigella* spp. and *Giardia duodenalis* from soil ingestion.

Current national estimates of sanitation access in the US rely on household survey data and exclude people experiencing homelessness. We improved on the current approach by combining household survey data from the US Census with data on homelessness from the Department of Housing and Urban Development to estimate that at least 930,000 urban Americans lack access to at least basic sanitation. To further explore the worst-case sanitation access in the urban US, we systematically surveyed open defecation sites across

a predefined 2.4-square-kilometer area in central Atlanta. At each site we recorded the GPS location, noted sanitary characteristics, and collected samples of fresh stools when possible. To identify the potential sanitary risks posed by open defecation, we tested stools for 15 enteric pathogens using a qualitative multiplex molecular assay.[3,4] We identified 118 human stools in our search area and of the 26 fresh stools we collected, 23% (6/26) tested positive for at least one enteric pathogen. Results suggest open defecation is common in Atlanta and may pose risks to public health. This was the first systematic survey of open defecation and related sanitary risks in the US.

The Maputo Sanitation (MapSan) trial was a controlled trial to evaluate the impact of a shared sanitation intervention on children's health in low-income urban neighborhoods of Maputo, Mozambique. We collected data from participants enrolled in the MapSan cohort and environmental samples taken in the domestic environment to evaluate the intervention's impact on pit-emptying practices, assess the potential of fecal sludges for pathogen surveillance, investigate the intervention's impact on enteric pathogens in soils, and estimate infection risks from soil ingestion.

Twenty-four months following the intervention we surveyed participants from intervention and control compounds (household clusters sharing sanitation and outdoor living space) regarding their pit-emptying practices. Likely due to the recent construction of the intervention, emptying an on-site sanitation system in the previous year was more frequent at control compounds (30%, [74/247]) compared to intervention compounds (5.6% [15/270]). Despite this infrequent emptying, among the subset of compounds that had emptied in the previous year we found intervention compounds were more likely to have hygienically emptied (aRR 3.8, 95% CI: 1.4, 10) than control compounds. Results suggest

that the construction of subsidized pour-flush sanitation systems increased hygienic emptying of fecal sludge in this setting. Though, further gains in hygienic emptying in urban Maputo may be limited by affordability and physical accessibility.

At the same time – 24-months following the intervention – we collected 95 stool samples from children enrolled in the MapSan trial and within 10 days we collected matched fecal sludge samples from the enrolled household's pit latrine or septic tank. We analyzed samples for 20 common enteric pathogens via multiplex qPCR. Among the 95 stools matched to fecal sludges, we detected the six most prevalent bacterial pathogens and all three protozoan pathogens in the same rank order in both matrices; we observed the same trend among both pit latrines and septic tanks. Our results suggest that sampling fecal sludges from on-site sanitation offers potential for localized pathogen surveillance in low-income settings where enteric pathogen prevalence is high.

In addition, we collected 179 soils at baseline (e.g. before the intervention) and at the 24-month phase from the domestic environment of control (n=91) and intervention (n=88) compounds. Similar to fecal sludges and stools, we tested soils for 20 common enteric pathogens including 10 bacterial pathogens, 5 viruses, 3 protozoa, and 2 soil-transmitted helminths (STH). Using a difference-in-difference analysis, we found evidence the intervention reduced the prevalence of any bacterial pathogen (aPR = 0.67, 95% CI: 0.44, 0.99) and the number of bacterial pathogens (aPR = 0.58, [0.34, 0.97]) in soils 24-months following the intervention, but had no effect on the prevalence or number of pathogenic viruses, protozoa, and STHs. Results suggest the intervention may have reduced the spread of some fecal contamination into the environment, but some pathogens remained widely prevalent in soils 24-months following the intervention. There are many potential reasons

the intervention had a limited effect on the spread of enteric pathogens into soils. The intervention did not address animal feces[5] and it did not attempt to achieve any threshold of sanitation coverage in neighborhoods with high population densities[6]. In response to the limited environmental impact and widespread detection of pathogens in soils at the 24-month phase, we characterized children's risk of infection from soils contaminated by *Shigella* spp. and *Giardia duodenalis* using a stochastic quantitative microbial risk assessment model (QMRA). We found that soil ingestion may be a substantial pathogen transmission pathway in low-income Maputo. More comprehensive WASH interventions – potentially complemented by other informal settlement upgrading strategies[7] – at a community level may be necessary to drastically reduce the spread of fecal contamination into the environment in similar settings.

CHAPTER 1. INTRODUCTION

The United Nations (UN) declared access to safe water and sanitation human rights in 2010.[8] As part of Sustainable Development Goal (SDG) 6, UN member states committed to pursue universal safe drinking water and adequate sanitation by 2030.[2] Responsible for monitoring progress towards SDG 6, the World Health Organization (WHO)/United Nations Children's Fund (UNICEF) Joint Monitoring Programme for Water Supply, Sanitation and Hygiene (JMP) defines a safely managed water service as "one located on premise, available when needed and free from contamination", and safely managed sanitation as a facility "where excreta is safely disposed *in situ* or treated off-site". Between 2000 and 2017 the percentage of people worldwide using safely managed water services increased from 61% to 71%, and safely managed sanitation services increased from 28% to 45%.[2] Although substantial progress has been achieved, inequities remain: the greatest progress often occurred among high income groups, and therefore combined national estimates often hide less progress among those with the lowest incomes. Indeed, due to population growth the total number of people without basic sanitation (improved sanitation infrastructure) in Sub-Saharan Africa (SSA) increased from 497 million in the year 2000 to 708 million in 2017. Likewise, in urban Mozambique, basic sanitation coverage increased from 32% in 2000 to 52% in 2017, but the gap between the richest and poorest quintiles increased by 30 percentage points.[2] In addition, demographic trends in SSA – where growth and migration contribute to an increasing urban population – will put increasing strain on already limited infrastructure.[7,9,10]

Inequalities in water, sanitation, and hygiene (WASH) access are not limited to low-income countries. In the US between 1900 and 1940 there was dramatic investment in large water

and sanitation infrastructure improvements, which were linked to substantial decreases in overall mortality rates.[11] However, this progress has been unequal and disparities remain.[12] In particular, people experiencing homelessness are an especially challenging population to provide with access to WASH services. People experiencing homelessness in unsheltered locations, which are locations unfit for human habitation such as in cars or under overpasses, do not have consistent access to running water and flush toilets. In fact, the number of people experiencing homelessness increased each year from 2016-2019[13], primarily driven by increasingly unaffordable housing costs in many US cities.[14] Without adequate access to WASH services, people experiencing homelessness were linked to large nationwide outbreaks of Hepatitis A virus from 2017-2020[15] and SARS-CoV-2 in 2020[16]. UN special rapporteurs to WASH and housing have repeatedly compared the conditions for people experiencing homelessness in the urban US to low-income informal settlements globally.[14,17–19] As current national estimates of water and sanitation access rely on housing survey data, it is crucial to generate robust national estimates of those without access. Such data can inform policy decisions in countries where resources exist to address inequalities.

To achieve safely managed sanitation, piped sewerage to offsite treatment remains a long-term goal for high population density urban areas. However, the construction and maintenance of such systems in the near term may not be affordable for low- and middle-income countries (LMIC) and is often complicated in urban informal settlements by unclear land tenure, and high population and housing densities that limit access for construction.[9] In addition, reticulated systems, reliant on a large and consistent supply of water, may not be sustainable or desirable as climate change is predicted to exacerbate water scarcity in some regions.[20,21] Where sewerage does not exist, safely managed

sanitation may take the form of on-site facilities that can be safely covered and abandoned when full, or that store excreta temporarily, emptied when necessary and then the fecal wastes must be transported for off-site treatment. This process – the sequestration, emptying, transport, treatment, and disposal or reuse of fecal sludge from pit latrines and septic tanks – is referred to as fecal sludge management (FSM).[22] In low-income urban areas with high population densities where space is limited for new on-site sanitation construction, hygienic FSM is necessary to ensure to the safe management of fecal wastes.

The primary goal of sanitation – including sewers and on-site sanitation systems – is to serve as a barrier between people and feces.[23] Enteric pathogens are the pathogenic bacteria, viruses, protozoa, and STHs that are excreted in human and some animals feces, and upon ingestion by a new host are capable of causing a new enteric infection. Enteric infection is the invasion of the intestines by a disease-causing agent, which reproduces, replicates or produces eggs/cysts, and the agent itself or the toxins it produces may cause a reaction by the host tissue. Notably, ingestion of enteric pathogens can lead to infection, with or without diarrheal disease[24], and a range of hypothesized sequelae including adverse growth outcomes[25] and cognitive impairment[26], detrimental impacts of the immune system[27], and reduced oral vaccine efficacy.[28] Interventions to prevent these adverse health outcomes often target well understood fecal-oral transmission pathways (Figure 1).[23] These pathways are represented by the "F-Diagram" and depict the spread of pathogens from feces to fluids (e.g. drinking water), fields (e.g. domestic soils, floors, or fields where food is grown), flies, fingers, or food before subsequent ingestion by a new host.

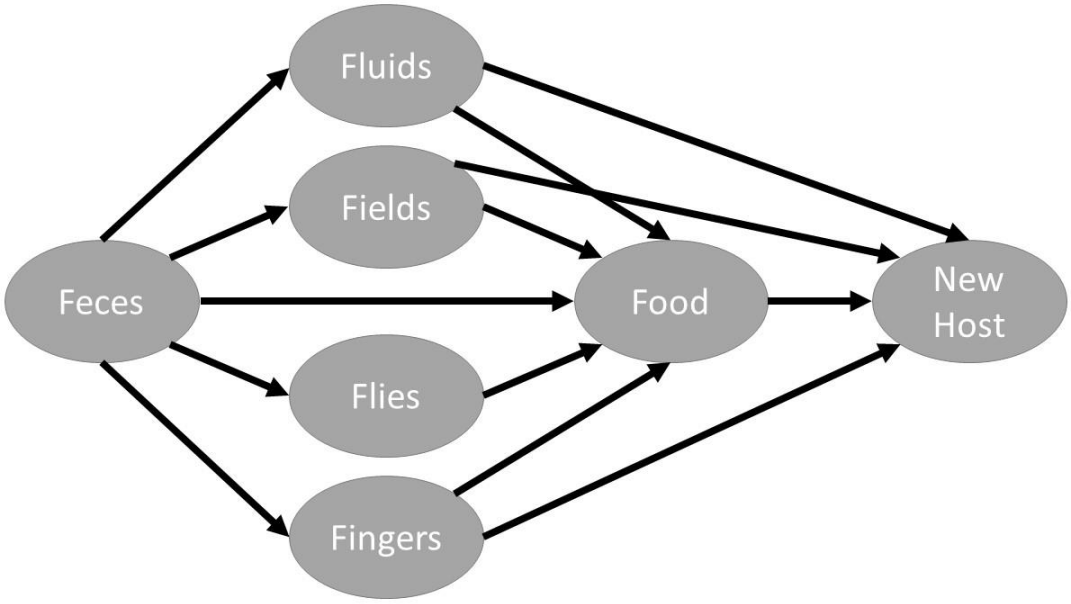

Figure 1. The F-diagram of enteric pathogen disease transmission. Adapted from Wagner and Lanoix, 1958.[23]

Analysis of environmental samples may indicate how WASH interventions interrupt the spread of feces and may be useful to evaluate and inform future interventions. It is possible to assess environmental fecal contamination in a variety of ways including sanitary surveys[29–33] that interpret responses to questionnaires or visually observe sanitary conditions, culture based methods that multiply microbial organisms or viruses by letting them reproduce in a predetermined culture medium under specific environmental conditions to potentially isolate an etiological agent[34], or molecular assays that test for the presence or concentration of a specific genetic sequence[35].

Sanitary surveys are questionnaire- and observation-based tools that assess sanitary conditions at household, neighborhood, or citywide scales. Such approaches to characterizing sanitary conditions are helpful to identify which areas are in greatest need of improvement and can inform the development of city sanitation master plans.[29–33]

Though the association of such tools with objective measures of environmental fecal contamination is not well characterized in urban settings (Appendix E).

Culture based assays that test for fecal indicator bacteria (FIB) – a proxy for enteric pathogens – or other culturable microorganisms have often been used due to their low cost, ease of use, and ability to indicate the viability of an organism. However, culture-based methods can be slow, require significant manual processing, are often used to test for primarily commensal FIB, methods do not exist to culture all microbes of interest, and they may miss viable-but-not culturable organisms.[36]

Molecular based assays are in some ways advantageous to cultured based methods because they produce results is less time, offer higher sample throughput, can be multiplexed to test for multiple targets simultaneously, and can directly measure enteric pathogens. On the other hand, molecular assays assess gene targets and not viability or infectivity. When feasible, the combination of culture- and molecular-based assays may offer nuanced insight into the spread of fecal contamination in the environment and the risks from exposure. Though, other methods such as staining with ethidium monoazide, which inhibits the amplification of DNA in dead cells during PCR, can be used to assess viability.[37]

Large-scale, rigorous studies have investigated the impact of WASH interventions on children's health by measuring the impact on diarrhea[38–42], growth[38–40,42], enteric infection[43–48], or intestinal biomarkers of gut permeability and inflammation as proxies for environmental enteric dysfunction[43,49], a subclinical and poorly understood condition which changes the structure and function of the intestines[50]. Some of these studies of WASH improvements conducted analysis of environmental samples to assess the impact

of the intervention on the spread of fecal contamination into the environment and along causal pathways that may contribute to enteric infections.[51–54] A 2016 meta-analysis – of studies that almost exclusively measured FIB – found improved sanitation in LMICs had no effect on the spread of fecal contamination into the environment. Sanitation interventions may only partially block the spread of fecal contamination to the environment, or the spread of fecal contamination through other pathways not associated with improved sanitation may explain the absence of an effect. However, some FIB such as *E. coli* may be naturalized in soils[55,56], which could prevent a study from detecting an effect on *E. coli* in soils.

One particularly rigorous study, the Water, Sanitation, and Hygiene Benefits (WASH-B) trial, was a large randomized controlled trial with study sites in rural Bangladesh and Kenya that implemented individual and combined water treatment, sanitation, handwashing, and nutrition interventions. In rural Bangladesh after one and two years of intervention, WASH-B found both the combined WASH arm and water treatment alone arm reduced the prevalence of *E. coli* in stored drinking water by 50% and the concentration of *E. coli* in stored drinking water by 1-$\log_{10}$. Furthermore, WASH-B Bangladesh observed a 30% reduction in *E. coli* prevalence and 0.5 $\log_{10}$ decrease in *E. coli* concentration in food at households that received the single water treatment and single handwashing intervention.[51] However, WASH-B Bangladesh did not observe a reduction of *E. coli* in groundwater, on child hands, or on objects. Likewise, in rural Kenya after two years of intervention, WASH-B observed a 19% reduction in *E. coli* prevalence in stored drinking water from water treatment alone and a 24% reduction from the combined WASH intervention, but did not observe an impact on measures of *E. coli* on child hand's or sentinel objects.[53] In addition,

a molecular analysis of environmental samples from WASH-B Bangladesh found a reduction of microbial source tracking markers on children's and mother's hands, but the associations were not significant after correcting for multiple comparisons.

A separate rigorous trial, the Sanitation Hygiene and Nutrition Efficacy (SHINE) trial was a cluster-randomized trial that tested the individual and combined effects of improving infant diet and household WASH on children's health outcomes.[40] However, the SHINE trial did not evaluate the impact of the intervention on fecal contamination in the domestic environment.

Several potential reasons may explain why the evidence from these trials suggests that the interventions did not dramatically reduce environmental fecal contamination. For example, sanitation interventions may require longer time periods to reduce fecal contamination in the environment, higher levels of community coverage may be necessary, the interventions may not have adequately addressed animal feces, or the transmission of enteric pathogens may vary between sites based on local behaviors and WASH related practices and were not comprehensively addressed by the interventions.[57–59]

1.1 Sanitation gaps in the urban US

The 250 million people who live in the urban US predominantly rely on piped sewers connected to wastewater treatment plants and on-site septic tanks to meet their need for safely managed sanitation. Since the 1800s, the dramatic reductions in cholera and typhoid fever incidence offers evidence that safely managed sanitation infrastructure contributed to improved public health outcomes.[11] However, many individuals, families, and communities in the urban US still encounter barriers to gaining and maintaining access to

sanitation.[12,60] For housed urban individuals, these access issues may result from inadequate maintenance of existing facilities.[61] While for the unhoused, a lack of public toilets, discriminatory practices at publicly accessible toilets, and mental and physical illness may be barriers to consistent access.[62] In addition to urban areas, insufficient access to household piped water and sanitation infrastructure remains a challenge for some rural communities, such as in the Navajo Nation (New Mexico) where some residents get water from monthly deliveries and in the Black Belt of Alabama where some residents use "straight-pipe" to discharge untreated fecal wastes into their backyards.[60,63,64]

Inclusive national estimates of sanitation access are necessary for policy makers to understand the scope of the problem and allocate resources to improve access. Current national estimates of sanitation access rely on the American Housing Survey (AHS), an annual survey conducted by the US Census Bureau.[2,65] However, the AHS asks about the presence of sanitation infrastructure (e.g. sewer connection, septic tank, or outhouse), but not about the presence or functionality of the toilets connected to the infrastructure. Work by Dr. Matthew Desmond and colleagues on low-income housing and eviction in the urban US suggested that functioning sanitation facilities are not universal in low-income urban housing units.[61,66] In addition, surveys such as the AHS only include housing units and do not consider people experiencing homelessness. My work aims to improve the existing methods used to estimate the number of people without access to at least basic sanitation – as defined by the UN – in the urban US (Chapter 2).

In fact, it is not well characterized what access the 120,000 people experiencing homelessness in unsheltered urban locations have to sanitation. It has been posited that the number of public toilets decreased in recent decades due to cost cutting measures and

cultural fears of illicit activity.[67] However, quantitative estimates of the availability of public toilets over time in the urban US are not available. Where data exists, the evidence suggests a dramatic lack of public toilets; in the Skid Row neighborhood of Los Angeles an audit found there were only nine public toilets for nearly 2,000 unsheltered homeless people during night time hours and those toilets were often inaccesible.[68] Defecation is a biological necessity, and people defecate with different needs and abilities to do so at various times and places.[69] In response to these conditions, I aim to characterize the worst case sanitation access (e.g. open defecation) – and the potential sanitary risks from exposure to human feces – in a major US city by people experiencing homelessness (Chapter 3).

1.2 Sanitation gaps in urban areas of low- and middle-income countries

The ambitious UN Sustainable Development Goal (SDG) 6.2, which aims to achieve universal safely managed sanitation by 2030, requires consideration of each point in the fecal waste disposal chain. As the dominant fecal-oral transmission pathways for infants may occur in the compound environment[70], we investigated the impact of the intervention on the points in the fecal waste disposal chain relevant to the compound environment: sequestration of fecal wastes and emptying of on-site sanitation systems. Further, we investigated the potential for pathogen surveillance using fecal sludges sequestered in on-site sanitation systems which may offer insight into the enteric infections circulating in the community. Finally, we used a stochastic quantitative microbial risk assessment model to translate the observed microbial hazards into infection risks.

1.2.1 Safely managed sanitation

On-site sanitation systems may fill-up after a period of time and become unusable. In rural areas where space is plentiful, it is considered safe to cover a full pit and dig a new one nearby.[2] Alternatively, dual pit latrines – such as those used at the rural WASH-B Bangladesh site – allow for on-site treatment of fecal wastes in one pit while the other pit is being used, before re-use as fertilizer or disposal.[71] Cities in LMICs often have high population densities which increasingly prevents the covering of old pits and construction of new pits as a safe solution for safely managed sanitation. Considering this reality, it was estimated that achieving safely managed sanitation will require FSM for at least 1.8 billion people in LMICs.[72]

There is a paucity of research on effective FSM interventions in LMICs where poor sanitation in commonplace. A 2014 analysis of fecal waste flows in 12 major cities in Africa, Latin America, and Asia indicated widespread challenges at each step in the fecal waste disposal chain, that is the sequestration, emptying, transport, treatment, and re-use or disposal of fecal sludges.[1] Poor construction quality of on-site sanitation systems adversely impacts the capacity for households to hygienically empty their systems. The walls of unlined pit latrines may collapse during emptying and dry systems often produce thick sludge that requires manual, and not mechanized, emptying.[22,73] Indeed, some evidence suggests that households may be more likely to hygienically empty pour-flush systems with septic tanks compared to pit latrines.[74] A lack of regulation – or the capacity to enforce existing regulations – often creates an enabling environment where pit emptiers face little to no repercussion for indiscriminately dumping fecal waste into the environment. In fact, in the limited instances where hygienic emptying and transport occurs, many cities lack FSM treatment facilities, and where facilities exist, fecal sludge is

often dumped into wastewater treatment plants which may result in the failure of the treatment process.[75] Overall, Peal *et al*. 2014 estimated less than one-third of fecal waste produced in the 12 cities they studied was safely managed.[1]

Considering these difficulties, the primary goal of on-site sanitation interventions in low-income urban settings should not be limited to the hygienic sequestration of feces but also aim to increase the likelihood fecal wastes generated are safely managed once pit emptying becomes necessary. From a policy perspective, this suggests that SDG 1.4 which targets universal access to basic services, including basic sanitation (i.e improved sanitation infrastructure), is inadequate. Instead, the more ambitious SDG 6.2 which targets safely managed sanitation for all, is likely a better benchmark for LMICs because the spread of fecal contamination to the environment at any step in the disposal chain poses infection risks. In Chapter 4, I evaluate the impact of an on-site sanitation intervention on pit emptying practices.

1.2.2 Surveillance

The results of the Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development (MAL-ED) study indicated that children's repeated exposures to and infection by enteric pathogens contribute to suboptimal growth in early childhood.[76] The Global Enteric Multicenter Study (GEMS) found that most cases of moderate-to-severe diarrhea were attributable to six pathogens (*Shigella* spp., rotavirus, adenovirus 40/41, STEC, *Cryptosporidium* spp., and *Campylobacter* spp.), but GEMS and other studies have indicated variations in the importance of these and other pathogens between sites.[24,43–48,77,78] In fact, in a consensus

piece on the implications of the results from recent WASH trials, Cumming *et al*. 2019 called for comprehensive WASH interventions "tailored to address the local exposure landscape and enteric disease burden".[57] Despite this evident need for site-specific data that characterizes the burden of microbial exposures, data is limited in many LMICs.

Current surveillance methods – where they exist – often rely on hospital or clinical patients who are typically symptomatic. Where stool-based testing occurs, samples may only be tested for a few pathogens. Conversely, sewage-based surveillance is increasingly used because it is relatively cheap, less invasive than stool collection, characterizes large communities that may not be included by conventional surveillance, does not require time consuming informed consent procedures, and may be sensitive to changes in disease incidence over time.[79–83] In the 1940s – before the development of the polio vaccine – sewage was helpful to estimate asymptomatic carriage of poliovirus in New York City.[82] Likewise sewage surveillance has been crucial in the global polio eradication campaign[83] and saw widespread use in 2020 to monitor the global spread of SARS-Cov-2, including reports that the SARS-CoV-2 RNA concentration in wastewater increased as the number of reported COVID-19 cases increased in a catchment area.[80,81,84,85] Indeed, the need for global data at a low-cost has led to a call for a global sewage surveillance system for anti-microbial resistance genes.[79]

In low- and middle-income cities – where current surveillance data is highly variable in quality and quantity – sewage surveillance offers potential to characterize circulating pathogens. However, piped sewerage often covers only a small portion of the population in LMICs and it often does not cover the lowest income communities where the burden of disease is highest.[78,86,87] For example in Maputo, Mozambique less than 10% of residents

are connected to the sewer network, and the most populous city in Mozambique – Matola – has no sewer network at all.[86,88] Adapting and validating existing sewage surveillance methods for LMICs where on-site sanitation systems predominate is a necessary step to develop scalable approaches that can be used to understand the local exposure landscape and burden of enteric disease. In Chapter 5, I aim to demonstrate the feasibility and potential to use fecal sludges as a surveillance tool for enteric pathogen transmission among children in low-income neighborhoods in Maputo, Mozambique.

1.2.3 Health impact assessment

Rigorous health impact evaluations of WASH interventions are useful to estimate the magnitude of health gains from a specific intervention in a specific context. If an effect is observed, environmental impact assessment may be helpful to elucidate how an intervention limited the transmission of enteric pathogens. Such results may help to improve our understanding of causal pathways and the potential risks from exposure to environmental matrices. Subsequently, these data may help improve and revise intervention strategies, which can be tested in future studies. Though external validity may be limited by the intervention and site-specific factors, the results of multiple trials across a variety of contexts may help stakeholders to develop locally relevant WASH policies.

However, due to the high cost of rigorous WASH trials[38–40], and since such trials have not demonstrated drastic improvements in health outcomes, an improved approach may be to first demonstrate an intervention's reduction in environmental fecal contamination, then commit additional resources to investigate a potential health impact. In fact, quantitative microbial risk assessment, which uses stochastic modelling to quantify infection risks from

microbial hazards, offers the capacity to assess what reduction in environmental fecal contamination would be necessary to achieve a certain reduction in infection risk.[89] Shorter and cheaper studies that evaluate environmental impact, rather than health impact, may allow the WASH field to identify effective interventions more rapidly.

The Maputo Sanitation (MapSan) trial is a controlled trial to evaluate a shared on-site sanitation intervention, and is located in densely populated, low-income neighbourhoods of urban Maputo, Mozambique where sanitary conditions are poor and the burden of disease is high.[90] In this and other low-income urban neighbourhoods shared sanitation by two or more households is often necessary due to a lack of space or money to build a private sanitation facility. On the WHO/UNICEF JMP sanitation ladder[2] – where the rungs are open defecation, unimproved, limited, basic, and safely managed sanitation – even if shared sanitation is well maintained and the fecal wastes hygienically emptied, due to its shared nature the WHO/UNICEF JMP still considers shared sanitation limited. Although, a 2014 systematic review found that households using shared sanitation were at increased risk of diarrheal disease and helminth infection.[91] Considering this association, but the lack of viable alternatives for some low-income urban areas, additional work is needed to evaluate if high quality shared sanitation facilities may be considered basic on the WHO/UNICEF JMP sanitation ladder.

The primary component of the intervention consisted of pour-flush latrines (ceramic squat plates or pedestals) to septic tanks with soakaway pits to discharge liquid effluent.[92] The sanitation infrastructure was shared (but not publicly accessible) and was designed to be used by multiple households in a compound (i.e. clusters of two or more households sharing sanitation and outdoor living space that typically has a wall or fence to delineate

property boundaries). Depending on the number of people sharing sanitation, Water and Sanitation for the Urban Poor (WSUP) – the non-governmental organization which implemented the intervention – built two different latrine designs. At large compounds with more than 20 members, WSUP built communal sanitation blocks (CSBs), which included a latrine stall for every 20 compound members, covered vent pipes for fly control, secure doors with padlocks, a municipal water supply connected to an elevated water storage tank (to allow for semi-continuous water access), a rainwater harvesting system and storage basin with floor level taps, a sink that could be connected to the water supply (connection not performed by WSUP), and a laundry facility. Smaller compounds with fewer than 20 members received shared latrines (SLs), which included a single latrine stall, a covered vent pipe, and a secure door with a padlock. WSUP constructed one latrine stall for every 20 members because this is the threshold set by the United Nations High Commission for Refugees for sanitation access.[93]

Twelve months following the sanitation intervention, Holcomb *et al*. 2020 studied microbial source tracking markers (MST) in source and stored water, food preparation surfaces, and soil collected at latrine and household entrances.[94] MST assays targeted fecal contamination overall (EC23S), from humans (HF183, Mnif) and from poultry (GFD). Results indicated that environmental fecal contamination was pervasive and no effect of the intervention on MST markers in environmental matrices was observed. However, the relatively low sensitivity and specificity of the HF183 (sensitivity = 0.64, specificity = 0.67) and Mnif (sensitivity = 0.71, specificity =0.70) assays observed by Holcomb *et al*. 2020 suggests that testing for enteric pathogens with more sensitive and specific assays[95] would be useful. In addition, because fecal contamination spreads to the environment

through many diverse pathways, the potential reduction in fecal contamination from the pathways blocked by the intervention may require a longer follow-up period to observe an effect, such that a two-year follow-up may have greater statistical power than the one-year follow-up conducted by Holcomb *et al*. 2020. My work aims to evaluate the impact of the intervention on pathogens in latrine entrance soils 24-months following the intervention (Chapter 6).

1.2.4 Quantitative microbial risk assessment

The widespread detection of fecal indicator bacteria[96–99], enteric pathogens[54,100,101], and microbial source tracking markers[54,94,101] from soils in LMICs suggests the infection risk from soil ingestion[102] may be high. In fact, there is evidence to suggest that in some settings children ingest a greater quantity of fecal indicator bacteria from soil compared to drinking water.[103] Although ingestion of small quantities of soil may be common by young children in LMICs, the probability of any single dose resulting in an infection is probably low. Quantitative microbial risk assessment (QMRA), a framework for translating microbial hazards into infection risks, is a useful tool to characterize such low frequency outcomes.[104]

While numerous studies have used QMRA to estimate infection risks from drinking water[105,106] and recreational activities[107–109], few have investigated the infection risks posed by soil ingestion in LMICs.[110,111] In fact, previous soil-focused QMRA models applied to LMICs were not stochastic[110,111], assumed 100% pathogen viability[110], assumed a large amount of soil ingested per dose[111] (e.g. five grams of soil), or did not include a sensitivity analysis[110,111]. A stochastic approach that accounts for these model parameters may result in an improved risk estimate. In addition, comparison with infection prevalence data from

children enrolled in the MapSan trial offers a unique opportunity to demonstrate the plausibility of infection from soil ingestion. In Chapter 7 I estimate children's infection risks from soil ingestion by *Shigella* spp. and *Giardia duodenalis* using a stochastic QMRA model.

CHAPTER 2. WATER AND SANITATION IN URBAN AMERICA, 2017-2019

Citation for the accepted manuscript:

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2.1 ABSTRACT

Objective: To estimate the population lacking at least basic water and sanitation access in the urban United States (US).

Methods: We compared national estimates of water and sanitation access from the World Health Organization / United Nations Children's Fund Joint Monitoring Program with estimates from the US Department of Housing and Urban Development on homelessness and the American Community Survey on household water and sanitation facilities.

Results: We estimate at least 930,000 urban Americans lacked sustained access to at least basic sanitation and 610,000 to at least basic water access, as defined by the United Nations.

Conclusions: After accounting for those experiencing homelessness and sub-standard housing, our estimate of people lacking at least basic water equaled current estimates (610,000)—without considering water quality—and greatly exceeded estimates of sanitation access (28,000).

Policy Implications: Methods to estimate water and sanitation access in the US should include people experiencing homelessness and other low-income groups, and specific policies are needed to reduce disparities in urban sanitation. We recommend similar

estimation efforts for other high-income countries currently reported as having near universal sanitation access.

2.2 INTRODUCTION

People experiencing homelessness and housing instability in towns and cities in the United States of America (US) may have limited or no access to safe water and sanitation. Water and sanitation are important to prevent infection by fecal-oral pathogens via well understood pathways of transmission[23], necessary for handwashing which may limit the spread of SARS-Cov-2, and are critical for maintaining public health. The number of people experiencing homelessness increased from 2016-2019[112], but decreased investment in urban sanitation infrastructure has resulted in lower access to public toilets.[11] Limited sanitation access for people experiencing homelessness was linked to a nationwide outbreak of Hepatitis A Virus from 2017-2018.[113] People living in emergency shelters and transitional housing share sanitation facilities with others[114] and people in unsheltered locations may not have sustained access to water and sanitation facilities, causing some in both groups to resort to open defecation.[62,115] Work by Desmond *et al*. on low-income housing and the eviction crisis suggested functioning water and sanitation facilities are not universal in low-income urban housing units in the US[61,116], in contrast to international statistics reporting universal or near-universal access.[2,117]

The United Nations Sustainable Development Goal (SDG) 6 calls for adequate and equitable sanitation, hygiene, and safe and affordable drinking water for all by 2030.[118] Under SDG 6, "safely managed" sanitation is defined as the, "use of improved facilities that are not shared with other households and where excreta are safely disposed of *in situ*

or transported and treated offsite". Basic sanitation is defined as "use of improved facilities that are not shared with other households." Improved facilities include "flush/pour flush to piped sewer systems, septic tanks or pit latrines; ventilated improved pit latrines, composting toilets or pit latrines with slabs.[2]" Safely managed drinking water is defined as "drinking water from an improved water source that is located on premises, available when needed and free from fecal and priority chemical contamination.[2]"

The World Health Organization and United Nations Children's Fund Joint Monitoring Program (WHO/UNICEF JMP) collects and reports national-scale data on water and sanitation across countries[2,117], but estimates are limited by the data shared by individual countries. The WHO/UNICEF JMP uses the American Housing Survey (AHS) to estimate national water and sanitation access and the US Environmental Protection Agency's Safe Drinking Water Information System (SDWIS) to assess drinking water quality.[117,119,120] These data sources exclude people experiencing homelessness, estimated to have been 570,000 in 2019, and so national statistics overestimate access to water and sanitation in the US.

To further examine published estimates of universal or near universal (>99%) access to safely managed water and sanitation in urban areas of the US [2,117], we conducted a scoping study to (1) identify sources of nationally representative data on access to water and sanitation in the US and to (2) estimate the number of people without access to basic water and sanitation in the US, inclusive of housing instability.

2.3 METHODS

We accessed publicly available data representing people living in urban areas of the US who may have insufficient access to water and sanitation facilities compiled by representative agencies in the form of downloadable Excel files and interactive online tables. The 2017 AHS produced nationally representative estimates that included a question regarding sanitation access (Question: Public Sewer), but only reported data for the 15 largest metropolitan areas (representing 39% of the total urban population) and select states.[65,121]

Inaugurated in 2005, the American Community Survey (ACS) is an annual survey by the US Census Bureau with a typical participation of 3.5 million households per year.[122] The ACS is mailed to specific addresses and participants can choose to respond via a paper form or the internet. The ACS contains one question about the presence of a complete bathroom, which it defines as the presence of hot and cold running water, a flush toilet, and a bathtub or shower. Additionally, it asks about the presence of a complete kitchen, which it defines as the presence of an installed sink with tap water, a mechanical refrigerator and a stove or range oven with built-in burners. The 2013-2017 ACS five-year estimates included data for all 382 US metropolitan areas[122] (urban clusters with populations ≥50,000) representing an additional 150 million urban US residents compared to the 15 largest metropolitan areas assessed by the AHS; therefore we use the more comprehensive ACS for our own estimates of urban water and sanitation access in the US.[122]

The ACS relies on a ratio estimation procedure to transform survey response data into nationally representative estimates. Weights are assigned to each sample person record (to produce person estimates) or to each housing unit record (to producing housing unit estimates), and are used to compensate for differences in sampling rates across areas,

between the full sample and the interviewed sample, and between the sample and independent estimates of basic demographic characteristics.[123] We accessed the ACS 2017 five-year estimates public use microdata set (PUMS) data using the Integrated Public Use Microdata Series (IPUMS)[124] (https://ipums.org/), filtering out responses not in a metropolitan area or if the metropolitan status could not be assessed, and used the ACS "person weight" option to access national population estimates.[125] To account for the high non-response rate (42%) to the ACS question on the presence of flush toilet we applied the same response distribution from people who did respond to those who did not respond. The non-response rate for other questions was small (<3%) and no adjustment was used.

We accessed publicly available data[126] on homelessness from the 2019 Department of Housing and Urban Development's (HUD) point-in-time (PIT) count.[112] The PIT count is an annual survey conducted each January where volunteers physically count people experiencing homelessness in housed and unhoused locations across the US.[112] The 2019 PIT count was conducted in 397 Continuums of Care across all 50 states, Washington DC and US territories.[127] Counted persons are categorized as living in an unsheltered or a sheltered location. Unsheltered locations are considered unsuitable for human habitation, such as under an overpass, or in a car, abandoned building or urban camping. Sheltered locations may include emergency shelters or transitional housing programs. The PIT count represents a conservative estimate of homelessness; it is cross-sectional and volunteers only count people physically located during the count.[128] We matched ACS 2017 5-year estimates with PIT count data by state to generate national estimates of water and sanitation access.

2.4 RESULTS

2.4.1 Sanitation

In the 2019 WHO/UNICEF JMP report, basic sanitation access for urban Americans was estimated to be >99%, while limited sanitation, unimproved sanitation and open defecation were each <1%.[2] Data available online from the WHO/UNICEF JMP estimated 96% (250,000,000) of urban Americans used safely managed sanitation, 4.5% (12,000,000) used basic sanitation, <0.01% (28,000) used unimproved sanitation in 2017.[117] Limited sanitation and open defecation were reported as non-existent.[117] Analyzed by facility type, the WHO/UNICEF JMP reported 93% (250,000,000) of urban Americans had a sewered connection, 6.5% (17,000,000) relied on a septic tank, and no urban Americans used a latrine or other form of sanitation.[117]

The ACS estimated in 2017 there were 100,000,000 occupied housing units in the 382 metropolitan areas of the US Census, totaling 250,000,000 housed urban people.[122] A small proportion of (0.30%, [750,000]) housed urban residents lacked a complete bathroom, defined as hot and cold running water, a bathtub or shower, and a flush toilet.[122] Hot and cold running water was most commonly absent from incomplete bathrooms (0.21%, [540,000]), followed by lack of a flush toilet (0.19%, [470,000])—which we adjusted for non-responses—and the absence of a bathtub or shower (0.18%, [460,000]).[122]

Very low-income households were most likely to report lack of access to a flush toilet; 0.37% (150,000) of people in households with incomes less than 100% of the national poverty threshold, as defined by the Social Security Administration, lacked a flush toilet, compared to 0.21% (90,000) of households with incomes from between 100% and 200% of the poverty threshold, and 0.14% (235,000) of households with incomes above 200% of

the poverty threshold. Additionally, a greater prevalence of people in renting households (0.26%, [220,000]) lacked a flush toilet compared to respondents who reported owning their unit (0.14%, [230,000]).[122] The ACS did not capture the number of flush toilets per households or what alternatives existed in the absence of flush toilet.

2.4.2 Water

The data reported by the ACS and WHO/UNICEF JMP are not directly comparable due to methodological limitations. To generate national estimates of water access, WHO/UNICEF JMP used data on water access and water quality, but the ACS only included data on water access.

The 2019 WHO/UNICEF JMP report estimated >99% of urban Americans had safely managed piped drinking water into their home and <1% had non-piped water access.[2] Data from 2017 estimated that >99% (270,000,000) urban Americans had a safely managed drinking water service, 0.11% (280,000) had basic service, 0.24% (610,000) had unimproved drinking water, and limited service or use of surface water was non-existent.[117] By facility type, WHO/UNICEF JMP estimated >99% (270,000,000) of urban Americans had access to improved piped water and 0.18% (460,000) had access to improved non-piped water, with no other service types reported.[117]

According to the ACS, a similar number of urban Americans lacked a sink with tap water (0.18%, [440,000]) compared to those lacking a flush toilet. The prevalence of not having an installed sink with tap water decreased with increasing household income: 0.29% (120,000) of people in households making <100% of the federal poverty threshold did not have a tap, decreasing to 0.23% (97,000) of people in households 100%-200% of the

poverty threshold, and an estimated 0.14% (230,000) of people in households >200% of the poverty threshold lacked a tap.[122] Additionally, the lack of a tap was more prevalent among renters (0.26%, [240,000]) compared to homeowners (0.13%, [210,000]).[122] The ACS did not capture what households used for drinking water in the absence of a sink with a tap.

2.4.3 Homelessness

On a single night in January 2019, the PIT count recorded 570,000 people in the US as experiencing homelessness.[112] Most (330,000) Americans experiencing homelessness were counted in urban areas; almost two-thirds (210,000) in urban areas were counted in sheltered locations and the remaining one-third were counted in unsheltered locations (120,000).[112] Additionally, about one-quarter (140,000) of people experiencing homelessness were counted in suburban areas, which included people counted up to 10 miles from urbanized areas.[112] Similarly, two-thirds (89,000) of the suburban homeless were counted in sheltered locations and one-third (46,000) in unsheltered locations.[112] The 2019 PIT count did not capture data on water and sanitation access for people experiencing homelessness.

2.4.4 Combined Data

Because people experiencing homelessness in unsheltered urban locations do not have a bathroom when and where it is needed[115,121] and those in sheltered locations generally use shared sanitation[114], we estimate that at least 0.29% of urban Americans (930,000) lacked access to at least basic sanitation in the urban US. This estimate is substantially greater

than the 0.01% of urban Americans (28,000) that WHO/UNICEF JMP reported as having limited sanitation, unimproved sanitation or resorting to open defecation (Table 1).

Assuming that people experiencing homelessness in sheltered urban locations have access to drinking water from an improved source for which the collection time is not more than 30 minutes, then those in sheltered locations should be considered to have access to basic drinking water. Those experiencing homelessness in unsheltered locations, however, may not have consistent access to an improved water source and/or their collection time may exceed 30 minutes; people experiencing homelessness and residing in unsheltered locations should therefore be considered to have limited or worse access to drinking water. When we combined data on homelessness with ACS data, we estimated that 610,000 (0.24%) of urban Americans lacked basic water access, without considering water quality.

Table 1. Estimated persons lacking at least basic access to water and sanitation services in urban US.

Urban Americans who:	WHO/UNICEF JMP 2019 estimates	Combined ACS & PIT Count Estimate (2017-2019)	2017 5-year ACS estimates	2019 PIT Count*
Lack at least basic water	610,000 (0.24%)	610,000 (0.24%)	440,000 (0.18%)	170,000 (0.07%)
Lack at least basic sanitation	28,000 (0.01%)	930,000 (0.37%)	470,000 (0.19%)	460,000 (0.18%)

*Includes both people counted in urban and suburban (<10 miles from urban) continuums of care.

The combined ACS and HUD PIT Count estimated urban residents did not have access to at least basic water and sanitation in every state except Wyoming (Table 2). Half of estimated urban residents without at least basic water (56%, [340,000]) and sanitation (50%, [470,000]) resided in four states: California, Florida, New York and Texas. Although

California comprises 12% of the total US population, 19% of all urban residents without at least basic water and 23% without at least basic sanitation resided in California.

Table 2. Estimates of inadequate access to water and sanitation by state

	Population (%) lacking access to at least basic:							
State	Sanitation	Water	State	Sanitation	Water	State	Sanitation	Water
AK	1,609 (0.54%)	1,130 (0.38%)	KY	4,582 (0.25%)	3,138 (0.17%)	NY	131,218 (0.74%)	43,848 (0.25%)
AL	4,644 (0.15%)	3,522 (0.11%)	LA	7,209 (0.23%)	5,912 (0.19%)	OH	23,889 (0.27%)	17,266 (0.19%)
AR	14,624 (0.24%)	14,206 (0.23%)	MA	11,100 (0.19%)	10,072 (0.17%)	OK	5,420 (0.26%)	2,917 (0.14%)
AZ	12,587 (0.92%)	7,699 (0.56%)	MD	15,815 (0.29%)	10,286 (0.19%)	OR	16,580 (0.58%)	8,463 (0.3%)
CA	214,930 (0.57%)	174,803 (0.46%)	ME	20,243 (2.58%)	2,046 (0.26%)	PA	30,261 (0.29%)	19,127 (0.18%)
CO	14,128 (0.34%)	5,917 (0.14%)	MI	17,293 (0.24%)	10,434 (0.14%)	RI	2,770 (0.26%)	1,549 (0.15%)
CT	8,296 (0.24%)	6,080 (0.18%)	MN	10,189 (0.29%)	6,261 (0.18%)	SC	9,969 (0.26%)	6,891 (0.18%)
DC	8,181 (1.22%)	1,462 (0.22%)	MO	6,704 (0.65%)	2,486 (0.24%)	SD	397 (0.23%)	333 (0.20%)
DE	2,430 (0.26%)	2,049 (0.22%)	MS	11,532 (0.28%)	9,713 (0.23%)	TN	10,003 (0.24%)	6,482 (0.16%)
FL	51,103 (0.27%)	42,704 (0.23%)	MT	65 (0.06%)	19 (0.02%)	TX	68,666 (0.3%)	49,384 (0.21%)
GA	22,170 (0.31%)	15,457 (0.21%)	NC	15,770 (0.23%)	10,852 (0.16%)	UT	6,328 (0.26%)	4,638 (0.19%)
HI	7,517 (0.76%)	5,688 (0.57%)	ND	114 (0.07%)	64 (0.04%)	VA	4,704 (2.16%)	866 (0.40%)
IA	3,573 (0.42%)	1,881 (0.22%)	NE	3,375 (0.33%)	2,039 (0.20%)	VT	10,159 (0.17%)	9,393 (0.16%)
ID	18,237 (0.17%)	15,059 (0.14%)	NH	2,100 (0.38%)	1,100 (0.20%)	WA	25,367 (0.42%)	15,083 (0.25%)
IL	15,960 (0.35%)	6,879 (0.15%)	NJ	24,668 (0.28%)	19,863 (0.23%)	WI	2,075 (0.62%)	685 (0.20%)
IN	3,305 (0.25%)	2,385 (0.18%)	NM	6,760 (0.50%)	4,229 (0.31%)	WV	7,311 (0.19%)	6,178 (0.16%)
KS	4,217 (0.27%)	2,013 (0.13%)	NV	11,630 (0.45%)	9,634 (0.38%)	WY	0 (0%)	0 (0%)

2.5 DISCUSSION

Access to water and sanitation is reported as near universal in the urban US, but the human rights to water[8], sanitation[8], and housing[129] remain unmet for people experiencing

homelessness and those living in homes without adequate water and sanitation. The JMP reports 28,000 people in the urban US lack access to at least basic sanitation; however, when accounting for residents experiencing homelessness and residents in sub-standard housing, we find at least 630,000 are without sustained access to a flush toilet and a further 300,000 rely on shared sanitation. The 930,000 people without access to at least basic sanitation services in the urban US—while a low overall percentage—is a large absolute number in a high-income country where resources exist to address the issue.

In the urban US, the human right to improved water and sanitation may be best advanced through the lens of adequate housing as a human right[129]; universal water and sanitation likely will only be achieved when universal affordable housing and rapid re-housing exist. Investments in public sanitation are crucial for public health—especially given the reduction in public sanitation in recent decades[11]—and public sanitation is used by both housed and unhoused people. Although, affordable and adequate housing is likely the best option to end open defecation and improve water and sanitation access in the urban US. Acknowledging the US Census, ACS, and AHS consistently undercount people experiencing homelessness, the US Census Bureau could incorporate PIT count data to improve national estimates of water and sanitation access, which are subsequently shared with the WHO/UNICEF JMP.

The 2013 AHS – which asked about household sewage disposal – estimated a very small proportion (0.0037%) of the urban population in the largest US metro areas did not have public sewer access or use a septic tank, cesspool, or chemical toilet.[65] This suggests most urban Americans that reported the absence of a flush toilet in the 2017 5-year ACS likely relied on an outhouse or did not have sustained access to a functioning flush toilet at home.

If safely covered and abandoned, or emptied and treated, outhouses can constitute safely managed sanitation. Without sustained access to a flush toilet, some households may use a neighbor's facilities, public facilities (e.g. at work or at a gym) or may resort to open defecation.[62,115]

WHO/UNICEF JMP estimates of water access are not directly comparable to ACS and HUD data. WHO/UNICEF JMP data considers the use of a water source, the quality the water, and allows for communal water facilities to be considered a basic drinking water service. However, data from the ACS and HUD only considers the presence of a water source. Therefore, the HUD and ACS estimate of people without a tap is a conservative baseline of drinking water access in the urban US and suggests the WHO/UNICEF estimate of people with limited water or worse is likely insufficient. Well-publicized lead contamination of drinking water in Flint, Michigan and Newark, New Jersey are examples that piped water into the home does not necessarily guarantee safety. The US government could report water quality data along with HUD housing data to the WHO/UNICEF JMP to improve future estimates of urban safe drinking water access.

Some households may have piped water and a flush toilet, but these facilities may fall into disrepair and landlords may take weeks or months to provide the necessary repairs.[66] Intermittent water supply—prevalent in low- and middle-income countries—has been linked to elevated risk of waterborne illness.[106] Strengthening laws that protect tenants may be helpful to empower renters to obtain necessary repairs. For low-income homeowners, increasing awareness about and expanding funds disbursed by government housing repair programs (e.g. the Very Low-Income Housing Repair Program) may be useful to achieve universal access.

The WHO/UNICEF JMP is limited by what official government data it receives and the need to apply a consistent methodology across countries. Some countries have explicitly included transient groups such as refugees (e.g. Palestinian and Syrian refugees in Lebanon) or nomadic groups (e.g. Ethiopia) in survey data collection.[120] However people experiencing homelessness are undercounted in national surveys based on household units, and not explicitly included in any national estimate of water and sanitation access. Without housing, it is likely impossible to have consistent access to a flush toilet and piped water when and where they are needed.[68,121] Over 90% of open defecation sites in urban Atlanta were less than 400 meters from shelters and soup kitchens.[115] UN Special Rapporteurs to water, sanitation and housing have repeatedly compared the squalid living conditions for people experiencing homelessness in the US to some of the worst settlements in low-income countries.[14,17] Analogous to people experiencing homelessness in the US, residents of informal urban settlements globally may also be excluded from data reported to the WHO/UNICEF JMP.[9]Without sustained access, people experiencing homelessness in unsheltered locations should be classified as "unimproved" on the JMP service ladder for drinking water and "open defecation" for sanitation. Due to the shared nature of water and sanitation facilities in emergency shelters and transitional housing, water for people experiencing homelessness in sheltered locations should be considered "basic" and sanitation considered "limited". Safely managed sanitation also requires adequate treatment of fecal wastes. In some rural US communities, direct discharge of raw sewage into the household yard—referred to as "straight-pipe"—is common.[63] In urban and rural areas, failing septic tanks and sewer overflows are common.[130] The US EPA estimates 850 billion gallons of untreated wastewater and stormwater are released as combined sewer

overflows each year.[130] Adequate access to sanitation, accompanied by sequestration, and treatment of fecal wastes remain important to achieving universal safely managed sanitation across the US.

Access to relatively worse water and sanitation facilities was more prevalent among low-income households. More detailed data about water and sanitation facilities would be useful but overlaps significantly with housing status; future ACS surveys will not include the question on flush toilets due to the high non-response rate and its perception as invasive.[131] Our results suggest the ACS should reintroduce the question regarding the presence of a flush toilet. Without comprehensive data on flush toilets, other metrics may also be useful. Nearly one million households were evicted in the US in 2016[116], leaving their short-term access to water, sanitation, and housing unclear. After an eviction some may experience homelessness such as doubling up with friends or family, staying in an emergency shelter, or sleeping in an unsheltered location.[66,116] Over half a million people were counted in the point-in-time count, but the count did not attempt an annual estimate of homelessness. The methods used excluded people staying with friends or family and those in hospitals or jails.[128] In 2017, after considering people in county jails who had experienced homelessness at the time of their arrest, Houston estimated their actual number of people experiencing homelessness was 57% greater than they reported in the PIT count (from 3,605 to 5,651).[132] A 2001 study estimated annual rates of homelessness in the US are 2.5 to 10.2 times greater than the cross-sectional PIT count estimate.[133] In high income countries where housing instability and homelessness are drivers of inadequate water and sanitation conditions, national estimates derived from household data are insufficient to accurately estimate water and sanitation access. Future estimates could consider or be

reported alongside housing data (e.g. evictions or worst-case housing)[66,112,134] to ensure low-income households and people experiencing homelessness are accounted for.

Our analysis has several limitations. Embarrassment may have caused households lacking a flush toilet to respond less often about their sanitation status than households with a flush toilet, suggesting our estimates may be biased downwards. Water and sanitation access for people experiencing homelessness in sheltered locations varies depending on accommodation: some may share facilities with others, lack access to the facilities during daytime hours, lose access temporarily as a punishment, or may have consistent private access in some cases. The heterogeneity in access may bias our estimate of people sharing sanitation.

Current data received by the WHO/UNICEF JMP from the US government excludes large groups in the urban US due to methodological limitations and data availability. The absence of a question regarding flush toilets in future ACS surveys suggests the WHO/UNICEF JMP should continue using the AHS, include HUD data on people experiencing homelessness, and consider other metrics of housing instability as proxies for water and sanitation access in the US. Including people experiencing homeless, a group often invisible to policy makers, in the data reported to the WHO/UNICEF JMP will enable the US to improve national estimates of water and sanitation, increase awareness of the issue, and allocate funding for investments in public toilets which could come from existing programs to improve stormwater quality.

2.5.1 Public Health Implications

Access to safely managed water and sanitation are human rights[8] and, without these basic services, people are at increased risk of infection by fecal-oral pathogens and SARS-Cov-2.[23] The absolute number of people excluded from basic water and sanitation access is higher than available international statistics suggest. Estimates that account for housing instability reveal disparities that require action. Our methods for estimating water and sanitation access may be applicable to other high-income countries.

CHAPTER 3. OPEN DEFECATION SITES, UNMET SANITARY NEEDS, AND POTENTIAL SANITARY RISKS IN ATLANTA, GEORGIA 2017-2018

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3.1 ABSTRACT

Objective: To survey the spatial distribution and enteric pathogen profile of discarded human feces in the city of Atlanta.

Methods: After defining priority search areas in central Atlanta, we conducted five searches of open defecation sites totaling 15 hours during the period from October 2017 to January 2018. We collected fresh stools for analysis via multiplex RT-PCR to identify presence of 15 common parasitic, bacterial, and viral enteric pathogens.

Results: We identified and mapped 39 open defecation sites containing 118 presumptive human stools; 23% of the 26 collected fresh stools tested positive for one or more pathogen. An estimated 12% of stools were positive for enterotoxigenic *E. coli* (ETEC), 7.7% for *Giardia* spp., 3.8% for norovirus, and 3.8% for *Salmonella* spp. The majority (92%) of identified open defecation sites were within 400 meters of a shelter or soup kitchen.

Conclusions Though constrained by a small sample size, results suggest that open defecation in Atlanta is common and may pose risks to public health.

3.2 INTRODUCTION

The association between open defecation (OD) and a wide range of health risks is well documented in low- and middle-income countries[135], as unsafely managed fecal contamination in the environment leads to potential for exposure to enteric pathogens. In high-income countries, OD can occur among persons experiencing homelessness who may lack ready access to sanitation facilities when and where they are needed.

An estimated 3 million people in the United States experience homelessness each year, with approximately one-third of counted persons occupying unsheltered locations.[136] Over 4000 people were estimated to be experiencing homelessness in Atlanta during the 2016 Point-in-Time Count, with nearly 1000 occupying unsheltered locations.[137] The ability of persons experiencing homelessness in the United States to maintain an adequate level of hygiene is constrained by the facilities available for their use, which may be highly variable across settings.[114] A reduced availability of public toilets in high-income countries in the past twenty years and the criminalization of homelessness through anti-nuisance laws has worsened sanitation access for some.[138,139]

Despite recent disease outbreaks associated with poor sanitation among persons experiencing homelessness – notably, HAV in California[140] – we identified no previous published studies of OD in the United States, either in terms of prevalence or associated microbial risks. In response to anecdotal accounts of OD in Atlanta by persons experiencing homelessness and references in the literature to the potential for OD caused by a lack of sanitation facilities serving this population,[140] we conducted an observational

survey to estimate the spatial distribution and enteric pathogen profile of discarded human feces in the city.

3.3 METHODS

We conducted a systematic search for OD sites in a pre-defined 2.4 km^2 area of central Atlanta, totaling 15 hours over five days during the period from October 2017 to January 2018. We mapped each site and counted all apparent human stools, also noting general site characteristics. We tracked the following indicators to distinguish between human and animal feces: presence of anal cleansing materials, makeshift latrines, presence of refuse such as paper and used condoms, presence of soiled clothing, multiple stools at an OD site, large volume stools, the presence or odor of urine, and characteristic patterns of feces resulting from defection while leaning against a surface.

When possible to do so without garnering attention from persons nearby, we collected a 2 g sample of fecal material. We sampled only apparently fresh, unfrozen stools with moist cores, from the centermost point of the stool. Within five hours of collection, samples were stored at -80^{o}C for later analysis. We extracted total nucleic acids from 100 mg of stool samples using the QIAamp 96 Virus QIAcube HT kit (Qiagen N.V., Hilden, Germany). We analyzed nucleic acid extracts through the xTAG Gastrointestinal Pathogen Panel (GPP) (Luminex Molecular Diagnostics Inc., Toronto, Canada). The Luminex GPP is a multiplex RT-PCR based method that detects common enteric pathogens, including: *Campylobacter* spp.; *Clostridium difficile*, toxin A/B; *E. coli* O157; enterotoxigenic *E. coli* (ETEC) LT/ST; Shiga-like toxin producing *E. coli* (STEC) stx1/stx2; *Salmonella* spp.; *Shigella* spp. *Vibrio cholerae*; *Yersinia enterocolitica*; adenovirus 40/41; norovirus GI/GII;

rotavirus A; *Giardia* spp. *Cryptosporidium* spp., and *Entamoeba histolytica*. The method is primarily used as a stool-based diagnostic assay that has been validated in comparison to other PCR-based methods for direct detection of enteric infections in a range of settings.[3]

3.4 RESULTS

We identified 118 discarded human stools in 39 sites in the search area. Of the OD sites, 85% appeared to offer some privacy or shelter; typical sites included overpasses (26%), adjacent to dumpsters (15%), narrow alleys with limited visibility (10%), and stairwells (8%). Other locations included parking garages, behind trees and bushes, behind buildings, in building alcoves, and along fences; the 15% of open locations were directly on or adjacent to sidewalks. We observed anal cleansing materials at 28% of OD sites, flies at 13% of OD sites; 92% of documented OD sites existed within 400 meters of a shelter or soup kitchen (Figure 2). We encountered 78% of stools at 33% of the OD sites.

Figure 2. Histograms showing distances from OD sites to the nearest shelter/soup kitchen or public restroom.

Of the 118 stools identified in the survey area, we analyzed the 26 stools with moist cores indicating recent disposal; 23% of these tested positive for one or more enteric pathogens: 12% tested positive for enterotoxigenic *E. coli* (ETEC), 7.7% for *Giardia* spp., 3.8% for norovirus, and 3.8% for *Salmonella* spp. Stool samples contained an average water content of 73% (SD = 10%), which is within the range of established values for fresh human stool.[141] We excluded one sample from analysis following inconclusive RT-PCR results. Random duplicate samples (n = 6) yielded fully consistent results.

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3.5 DISCUSSION

This study provides evidence that OD is relatively common in the urban core of a major American city, despite global statistics that suggest universal access to sanitation[142]. Although not a comprehensive survey of OD in Atlanta, we identified 39 OD sites containing 118 stools in a rapid search that was limited to 15 hours over 5 days. The number of stools encountered in this survey was limited by the presence of other persons: we purposefully avoided contact with people in the search areas, due to concerns about causing embarrassment. It is possible that not all stool was of human origin or from persons experiencing homelessness. Also, we cannot estimate the population of persons resorting to OD because multiple stools in this study may have been from the same individual.

It is likely that OD in Atlanta is not limited to the small area we surveyed. We did not search areas surrounding any apparent homeless camp, identified by the presence of tents or bedding, or any area marked "no trespassing." Excluding these sites introduces a potential bias in our counts of OD sites in the search area.

The proximity of OD sites to shelters and soup kitchens suggests those facilities are not meeting the sanitary needs of persons experiencing homelessness. Since 78% of the stools in this study were found at only 33% of the OD sites, we suspect that localized sanitation interventions might be effective at reducing OD. However, the use of sanitation facilities by persons experiencing homelessness is a complex issue that may be limited by systematic challenges such as safety, privacy, and accessibility.[62,114]

Further, molecular analysis of stools indicated presence of enteric pathogens, suggesting both that persons experiencing homelessness may be at relatively high risk of enteric infection and that there may be risks associated with exposure to discarded feces. An estimated 8.2% of the US population are infected annually with *norovirus*, 0.7% with *Giardia*, 0.5% with *Salmonella*, and 0.03% with ETEC[143], though prevalence in stools outside clinical settings has not been characterized in a comparable population. Molecular analyses in this study did not capture viability or infectivity of enteric pathogens identified, so estimates of exposure risks are not possible from our data.

For persons experiencing homelessness, limited access to sanitation facilities and resource constraints at existing facilities may present challenges to maintaining dignity, health, and privacy[114]; these basic human needs are among the reasons sanitation has been declared a human right[8]. Achieving universal sanitation access in the United States will require consideration of "invisible" populations whose needs are unmet and whose rights have not yet been realized.

CHAPTER 4. IMPACT OF AN INTERVENTION TO IMPROVE PIT LATRINE EMPTYING PRACTICES IN LOW INCOME URBAN NEIGHBORHOODS OF MAPUTO, MOZAMBIQUE

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Capone, D.; Buxton, H.; Cumming, O.; Dreibelbis, R.; Knee, J.; Nalá, R.; Ross, I.; Brown, J. Impact of an Intervention to Improve Pit Latrine Emptying Practices in Low Income Urban Neighborhoods of Maputo, Mozambique. *International Journal of Hygiene and Environmental Health*. 2020, 226.

4.1 ABSTRACT

Safe fecal sludge management (FSM) – the hygienic emptying, transport, and treatment for reuse or disposal of fecal sludge – is an essential part of safely managed sanitation, especially in towns and cities in low- and middle-income countries with limited sewer coverage. The need for safe and affordable FSM services has become more acute as cities grow and densify. Hygienic pit-emptying uses equipment that limits direct human exposure with fecal sludge and hygienic transport conveys fecal sludge offsite for treatment. We evaluated whether a program of on-site sanitation infrastructure upgrades and FSM capacity development in urban Maputo, Mozambique resulted in more hygienic pit-emptying and safe transportation of fecal sludge. We compared reported emptying practices among multi-household compounds receiving sanitation upgrades with control compounds, both from the Maputo Sanitation (MapSan) trial at 24-36 months after the intervention. Intervention compounds (comprising 1 – 40 households, median = 3) received a subsidized pour-flush latrine to septic tank system that replaced an existing shared latrine; control compounds continued using existing shared latrines. We surveyed compound residents and analyzed available municipal data on FSM in the city. Due to the recent

construction of the intervention, emptying was more frequent in control compounds: 5.6% (15/270) of intervention compounds and 30% (74/247) of controls had emptied their on-site sanitation system in the previous year. Among those compounds which had emptied a sanitation facility in the previous year, intervention compounds were 3.8 (95% CI: 1.4, 10) times more likely to have to done so hygienically. Results suggest that the construction of subsidized pour-flush sanitation systems increased hygienic emptying of fecal sludge in this setting. Further gains in hygienic emptying in urban Maputo may be limited by affordability and physical accessibility.

4.2 BACKGROUND

The United Nations Sustainable Development Goal (SDG) 6 calls for universal access to "safely managed" sanitation by 2030, defined as the "use of improved facilities that are not shared with other households and where excreta are safely disposed of *in situ* or transported and treated offsite" [144]. Piped sewerage and centralized wastewater treatment only serves an estimated 316 million people in cities of LMICs [72]. For many cities in LMICs, expansion of networked sewerage to growing populations can be cost-prohibitive [145] and some cities struggle to adequately maintain existing sewer infrastructure [88,145]. Expanding networked sewerage to unplanned settlements is often complicated by complex or unclear land tenure, high population and housing densities limiting access for construction, and an absence of city planning and basic infrastructure [9]. In addition, reticulated systems, reliant on a large and consistently reliable supply of water, may not be sustainable nor desirable [20].

When on-site sanitation systems—such as pit latrines or septic tanks—fill, fecal sludge must be either safely sequestered *in situ* or must be hygienically emptied, safely

transported, and adequately treated for reuse or disposal. As space becomes increasingly limited in densifying urban and peri-urban communities where on-site systems predominate, the common practice of covering and abandoning pits once full becomes unworkable (Figure A1). Decreasing space and the construction of more permanent latrine superstructures necessitate emptying as part of a safely managed fecal sludge management (FSM) service chain [1]. Hygienic emptying and transport of fecal wastes to a treatment plant are necessary to reduce exposures to fecal-oral pathogens, as repeated exposures to fecal-oral pathogens are associated with negative impacts on child health and survival [146,147]. Achieving SDG sanitation targets will therefore require providing safe and hygienic FSM services to at least 1.8 billion people who rely on on-site sanitation technologies and lack access to these services [72,86,148].

The Maputo metropolitan area contains 2.7 million people [149] but only 136,000 people are served by a sewer connection and most of the wastewater from the sewers discharges untreated into Maputo Bay [88,150]. Of the unsewered population, 36% of households use dry pit latrines and 64% use pour-flush latrines with disposal to pits or septic tanks [87]. Most households have a private on-site sanitation facility; 16% of on-site sanitation facilities are shared by two or more households [74]. Maputo has no designated treatment facility for fecal sludge. Instead, fecal sludge is discharged to anaerobic ponds at a nearby wastewater treatment plant (WWTP) according to municipal by-laws [74,151].

Pit-emptying businesses and organizations can pay an annual fee to formally register with Maputo Municipal Council (US$ 67-167) and a monthly fee to dispose fecal sludge at the WWTP ($25-$75) [151]. Fines for illegal dumping of fecal sludge range from $100-$167 USD, but it is unclear how commonly fines are imposed. Unhygienic informal pit-

emptying is illegal in Maputo but remains common across the low-income areas. An estimated 60% of emptying was performed by unhygienic emptiers in 2016 [74]. In 2013 it was estimated that 100% of fecal sludge from unhygienic emptying was disposed illegally in or near customers' yards, while 25% of fecal sludge from more hygienic emptiers was disposed illegally [74,152].

Pit-emptying practices can be categorized as hygienic or not based on plausible exposure risks to the community and those engaged in emptying and transportation [153,154]. Unhygienic pit-emptying typically uses manual equipment, such as buckets and shovels, and is often performed at night due to social stigma and the intense smell produced [155]; fecal sludge is often buried in the customer's yard or dumped nearby in a drain or ditch, which may contribute to the transmission of enteric pathogens through well-understood pathways [23,87,152–154]. Household members or manual laborers from the local community often perform unhygienic emptying.

Hygienic pit-emptying typically uses mechanized equipment (e.g. a trash pump or vacuum tanker) or manual pumps (e.g., a Gulper) [44,75,153,156] together with personal protective equipment (e.g. gloves, mask, boots, and work uniform). Mechanical emptying is hygienically preferable to manual emptying as it lessens the risk of contact with fecal sludge by both emptiers and residents, but some fecal sludge may still spread into the environment due to the aerosolization of microbes, the poor condition of hoses used to pump fecal sludge, dismantling of hoses after emptying, and inadequate cleaning of equipment [157,158]. Hygienic emptying is often performed by businesses and community-based organizations that may be registered with a local municipality to provide emptying services. These hygienic emptiers are more likely to transport fecal sludge to a treatment

plant than to bury or dump it near the customer's home, due to reputational risk or government regulations. Hygienic pit-emptying is an important first step in the FSM service chain.

In high-density unplanned settlements where space is unavailable to allow the covering and abandonment of full pits, the transport of fecal sludge can be categorized as hygienic or not by considering where the fecal sludge is deposited. In such settings, fecal sludge that is emptied and dumped on-site may pose greater pathogen transmission risks than excreta which is transported to a treatment plant [87,146,152]. However, it is difficult for customers to know where their waste is deposited once it leaves their property. Tipping fees, traffic, and long transport times may discourage emptiers from disposing of fecal sludge at treatment plants [75,159].

The objectives of our cross-sectional study in the *Nhlamankulu* and *KaMaxaquene* Districts of Maputo were to: (1) evaluate the effect of an on-site sanitation and FSM strengthening intervention on increased hygienic pit-emptying and transportation of fecal sludge and (2) identify key remaining barriers to the uptake of safe FSM practices.

4.2.1 The Maputo Sanitation (MapSan) Project

The Maputo Sanitation Project aimed to improve sanitation conditions and FSM for the residents living in the 11 low-income neighborhoods (*bairros*) of the *Nhlamankulu* District and 5 neighborhoods of the *KaMaxaquene* District in Maputo, Mozambique [92]. The project used an approach with three components: (1) construction of privately shared on-site sanitation infrastructure, (2) support for community based organizations (CBOs) and commercial micro-enterprises to provide desludging services and (3) community level

sanitation and hygiene promotion [92]. Component one targeted specific compounds (household clusters), while components two and three targeted neighborhoods in the *Nlhamakulu* and *KaMaxaquene* Districts. The program was funded by the Japanese Social Development Fund (JSDF); Water and Sanitation for the Urban Poor (WSUP) was responsible for implementing the sanitation infrastructure component, development of desludging services was a joint venture between WSUP and the Water and Sanitation Program (WSP) of the World Bank, and WSP was responsible for the community level sanitation and hygiene promotion [87].

Component one, the construction of sanitation infrastructure, consisted of subsidized provision of pour-flush toilets (to septic tank with a drain field) shared by multiple households in compounds (Appendix A.1 **Detailed description of the sanitation intervention**). Compounds with approximately 15-20 people received a shared latrine (SL, Figure A2)and generally compounds with ≥21 people received a community sanitation block (CSB, Figure A3). Compound residents were expected to contribute about 8-10% of the construction cost (compounds contributed on average $97 for a CSB and $64 for a SL per compound), but operation and maintenance costs, including pit-emptying, were not subsidized [92]. High-water table areas were excluded from receiving sanitation infrastructure to prevent water infiltration into the system sub-structure. Additionally, intervention systems were designed with the intention that future emptying would be performed hygienically with mechanized equipment every two years; access by a vacuum truck was a site criterion for community sanitation blocks (≤60 meters from a truck-accessible road) but was not considered for placement of the shared latrines. The Maputo Sanitation (MapSan) trial was a controlled, before-and-after trial of the Maputo

Sanitation Project component one that assessed the impact of the intervention on enteric infections and other health outcomes in children [78,90].

Component two, the development of hygienic emptying organizations, aimed to establish enterprises capable of delivering hygienic services, but there was no intention to ensure prices accessible to *Nhlamankulu* and *KaMaxaquene* District residents *a priori*. WSP identified eight members of a national waste management association operating in or near the *Nhlamankulu* and *KaMaxaquene* Districts and focused on providing the capacity to perform hygienic pit-emptying in addition to their existing services (typically weekly trash pick-up) [92]. Capacity building took the form of technical assistance, provision of emptying equipment, monitoring and evaluation of FSM services, a marketing program to promote hygienic emptying (e.g. television ads and flyers), technical development in the form of training to use the provided equipment for hygienic emptying, and training in business management.

Component three aimed to develop and support community-level sanitation hygiene promotion and monitoring activities through engagement with appointed local government officials (*chefes de quarteirão*) and community members [87,92]. Workshops were held with local stakeholders and institutions to build capacity and establish monitoring systems. Sanitation promotion activities were implemented that focused on encouraging households to improve their on-site sanitation systems [87]. We did not evaluate component three as it fell outside the scope of this study, only occurred three neighborhoods, and its results are reported elsewhere [87].

4.3 MATERIALS AND METHODS

4.3.1 Survey groups

We conducted surveys of on-site sanitation, revealed preferences of previous pit-emptying activities, and stated preferences for future pit-emptying activities. We trained enumerators to conduct the interviews through a two-day facilitated workshop and during one week of survey piloting in December 2017 and an additional two days of survey piloting in April 2018. All questionnaires were administered in Portuguese or the local language, Changana, as requested by the respondent. Enumerators verified sanitation infrastructure by direct observation, recorded each latrine's characteristics with illustrative photographs. Our sample frame included three primary respondents: caregivers of children enrolled in the MapSan trial; an additional respondent from the same compound who was not previously enrolled in the MapSan trial (Appendix A.2 **Method for identifying non-MapSan trial respondent.**), and compound leaders. Our sampling strategy was intended to maximize diversity among the respondents (Appendix A.2 **Method for identifying non-MapSan trial respondent.**).

In July 2018 we met with the Maputo Municipal Council's Department of Water and Sanitation (DAS). They provided us with a list of all registered emptying businesses in Maputo and their log of visits by the businesses to the Infulene WWTP to dispose of fecal sludge during the months of August, September, October, and November in 2017, and January and February 2018. DAS did not have digitized data available for December 2017 and we excluded this data from our analysis. We conducted all surveys and interviews from April – August 2018.

4.3.2 Data analysis

We analyzed data in R version 3.5.0 (R Foundation for Statistical Computing, Vienna, Austria). To account for clustering at the compound level we used generalized estimating equations (GEE) with the "exchangeable" correlation structure and a Poisson (log) distribution for calculation of adjusted risk ratios (aRR) [160–162]. For Poisson regression modelling we decided *a priori* to control for household wealth, the number of people who used the respondent's on-site sanitation system as their primary sanitation facility, and the respondent's gender and age as potential confounders [22,163,164]. Due to a small sample size for the intervention and the subset of control compounds with pour flush technology that had emptied their on-site sanitation system in the previous year, we used the non-parametric *Somer's d* test to determine the association of hygienic emptying with intervention status [165].

We calculated household wealth using eight of the ten inputs from the Simple Poverty Scorecard for Mozambique [166]. We excluded number of beds (limited data) and sanitation [167] from our calculation of household wealth. The compound leader is not a formal leadership role and was often the person resident in the compound for the longest time, therefore we did not calculate a household wealth score for compound leaders and instead used the average wealth scores of the other household respondents inside the same compound to adjust for wealth.

Recognizing that interviews with one to three individuals at each compound had the potential to skew survey response data, we report data from all survey respondents for stated preference questions (e.g. future intentions about emptying) and a single priority-based respondent from each compound for revealed preference questions (e.g. emptied an on-site sanitation system in the previous year). To select a single respondent to represent

each compound we assigned priority based on an assumption of which respondent type would most likely have access to accurate information. Therefore, for the revealed preference questions, we first analyzed the response from the compound leader, and, if no compound leader was interviewed, we used the response from the MapSan trial respondent. If neither a compound leader nor a MapSan trial respondent was interviewed, we used the response of the non-MapSan trial respondent as the compound response.

4.3.3 Ethical approvals

Before conducting a survey with a respondent, we obtained written informed consent. The study protocols were approved by the Comité Nacional de Bioética para a Saúde (CNBS), Ministério da Saúde (333/CNBS/14, 81/CNBS/18), the Ethics Committee of the London School of Hygiene and Tropical Medicine (Reference # 8345) and the Institutional Review Board of the Georgia Institute of Technology (Protocol # H15160, # H18027). The associated MapSan trial has been registered at ClinicalTrials.gov (NCT02362932).

4.4 RESULTS

4.4.1 Respondent characteristics

We visited 403 MapSan households, of which 399 (99%) consented, and 386 non-MapSan households, of which 378 consented (98%). We visited an additional 63 households which did not meet our eligibility requirements and were not included in this study (Appendix A.3 **Details regarding compounds that did not meet eligibility requirements**). The median amount of time respondents lived in their home was 15 years and the mean was 18 years. Compounds contained an average 4.3 families, 16 people, and 2.4 children under the

age of five. We recruited 300 *chefes de composto* from 149 control compounds and 151 intervention compounds, of whom 295 (98%) consented to participate.

4.4.2 Household and compound leader interviews

Respondents from intervention compounds primarily reported using shared latrines (78%) with the remainder using community sanitation blocks (22%). Respondents from control compounds primarily reported using pit latrines with (34%) or without a slab (29%); other control respondents reported using a septic tank or pour flush to an underground pit (30%) or above ground pit (7%). The quality of the on-site sanitation structure was generally better for intervention compared with control compounds, as measured by observable characteristics including building integrity and cleanliness. Enumerators observed that the slabs/floors of intervention systems were 1.6 (95% CI: 1.5, 1.8) times more likely to be in good condition with no cracks, holes, or visual structural defects compared to control systems. Reported educational visits by WSUP to discuss hygienic emptying were not common; about one-quarter of intervention compounds (27% [74/270]) reported ever receiving a visit from WSUP where hygienic pit-emptying was discussed compared to about one-eighth of control compounds (12% [30/247]), but respondents most frequently reported being unsure (Table A1).

Intervention household respondents more often reported a water tap inside the compound (68%) than control household respondents (57%) (Table A2). Control household respondents who reported having pour-flush sanitation more often reported a water tap inside the compound (69%) compared to those who did not report having pour-flush

sanitation (50%). Both intervention and control household respondents most frequently reported running water was available for 4-6 hours a day (Table A2).

Emptying of an on-site sanitation system in the previous year was less common in intervention compounds; 5.6% (15/270) of intervention compounds reported emptying in the previous year compared to 30% (74/247) of control compounds (Table 3). Most intervention compounds that reported emptying in the previous year were shared latrines (11/15) and some were community sanitation blocks (4/15). A one-quartile increase in household wealth was not significantly associated with previous year emptying at either intervention (aRR: 0.99, 95% CI: 0.73, 1.3) or control compounds (aRR: 1.1, 95% CI: 0.57, 2.2).

Among compounds where an on-site sanitation system was emptied in the previous year, hygienic emptying was more common at intervention compounds; 14% (10/74) of control compounds reported using a hygienic emptier compared to 73% (11/15) of intervention compounds (Table 1). All community sanitation blocks (4/4) and most shared latrines (7/11) which were emptied in the previous year used a hygienic emptier. No control compound reported using a pit-emptier who was equipped by WSP as part of intervention component two, while 20% (3/15) of intervention compounds reported using an intervention-supported pit-emptier. Adjusted for confounders, intervention compounds were 3.8 times (95% CI: 1.4, 10) more likely to have used a hygienic emptier in the previous year compared to control compounds.

Most hygienic emptying in control compounds occurred in a subset of those with pour-flush systems; at the 74 control compounds which reported emptying in the previous year,

36% (8/22) of those with pour-flush systems reported hygienic emptying compared to 3.8% (2/52) of those with dry pit latrines. For control compounds with pour-flush technology (n = 22) and intervention compounds (n = 15) that had emptied their sanitation system in the previous year, we used the unadjusted *Somer's d* statistic to characterize the association between intervention status and hygienic pit-emptying. We found a positive correlation between the presence of the intervention and hygienic pit-emptying in the previous year (d = 0.36, 95% CI: 0.06, 0.65).

Table 3. Responses to revealed preference survey questions from all respondents, 24-36 months following the intervention

	Priority Compound Respondent			Household Respondents*		Compound Leader	
	Control (n)	Intervention (n)	aRR (95% CI)	Control (n)	Intervention (n)	Control (n)	Intervention (n)
Compound emptied an on-site sanitation system in the previous year	30% (74/247)	5.6% (15/270)	0.17 (0.09, 0.29)	29% (108/378)	7.0% (28/399)	31% (40/129)	7.0% (10/143)
Compound constructed ≥1 new on-site sanitation system(s) in the previous 3 years (excluding the intervention systems)	34% (84/247)	15% (41/270)	0.39 (0.26, 0.58)	30% (111/378)	13% (51/399)	33% (42/129)	16% (23/143)
Any on-site sanitation system in the compound flooded in the previous year	7.3% (18/247)	4.8% (13/270)	0.71 (0.33, 1.5)	7.9% (30/378)	3.0% (12/399)	7.8% (10/129)	7.0% (10/143)
Responses to revealed preference questions from the subset of compounds that emptied an on-site sanitation system in the previous year							
Hygienic emptier was used for emptying in the previous year	14% (10/74)	73% (11/15)	3.8 (1.4, 10.0)	18% (19/108)	54% (15/28)	13% (5/40)	70% (7/10)
Pit was emptied mechanically in the previous year	9% (7/74)	67% (10/15)	4.5 (1.5, 14)	14% (15/108)	43% (12/28)	10% (4/40)	70% (7/10)
Emptied fecal sludge taken to WWTP or transported from the compound to an unknown location (e.g. not buried on-site, not buried outside the compound, or respondent was unsure)	3% (2/74)	40% (6/15)	30 (3.3, 270)	8% (9/108)	18% (5/28)	2.5% (1/40)	50% (5/10)
Compound used an intervention pit-emptier (from component two) for emptying in the previous year	0% (0/74)	20% (3/15)		0% (0/108)	21% (6/28)	0% (0/40)	30% (3/10)

*There were up to two household respondents per compound

Note: aRR: adjusted risk ratio. CI: confidence interval.

Similarly, at compounds where an on-site sanitation system was emptied in the previous year, intervention compounds reported their fecal sludge was taken by the pit-emptier to a WWTP or transported away by a vehicle to an unknown location more frequently than control compounds (Appendix A.4 **Detailed description of fecal sludge transport**); 2.7% of control compounds (2/74) reported their fecal sludge was transported away after emptying compared to 40% of intervention compounds (6/15). Adjusted for potential confounders, fecal sludge was 30 times more likely (95% CI: 3.3, 270) to have been transported away from intervention compounds compared to controls. Intervention and control compounds that used mechanized emptying reported their waste was transported to a WWTP or away (47%, [8/17]) more frequently than compounds that used manual emptying (0%, 0/72) (Table 4).

Table 4. Locations where fecal sludge was deposited

Reported destination of fecal sludge after emptying at compounds who emptied systems in the previous year (priority respondent)	**Control compounds**	**Intervention compounds**	**Compounds that used mechanized emptying**	**Compounds that used manual emptying**
Transported to WWTP	1.4% (1/74)	6.7% (1/15)	12% (2/17)	0% (0/72)
Transported away from compound to an unknown location	1.4% (1/74)	33% (5/15)	35% (6/17)	0% (0/72)
Buried on-site	77% (57/74)	27% (4/15)	0% (0/17)	85% (61/72)
Buried outside the compound	2.7% (2/74)	0% (0/15)	0% (0/17)	2.8% (2/72)
Dumped outside the compound	0% (0/74)	0% (0/15)	0% (0/17)	0% (0/72
Unsure	18% (13/74)	33% (5/15)	53% (9/17)	13% (9/72)

Respondents reported various methods for deciding when to desludge (Table A3). Intervention respondents most often cited smell (40%, [215/542]), control respondents with pour-flush systems most often cited a visual inspection of the fecal sludge level (32%, [58/180]), and control respondents without a pour-flush system were most often unsure

how they would decide (46%, [152/327]) (Table A3). Nearly one in five intervention respondents (19%, [101/542]) and control respondents with pour-flush systems (18%, [33/180]) stated they would decide to empty their sanitation system once it was overflowing (Table A3). Despite being designed for emptying every two years, few intervention respondents (8.3%, [45/542]) stated they would empty their system based on time in use.

Over half of intervention respondents (58%, [315/542]) indicated they would use a hygienic pit-emptier next time their latrine needs emptying, compared to 18% (89/507) of respondents from control compounds (Table A4). Most control respondents which stated a preference for future hygienic emptying possessed pour flush systems (Table A4). Adjusted for potential confounders, respondents from intervention compounds were 3.1 (95% CI: 2.4, 4.0) times more likely to express the intention to use a hygienic emptier and 0.54 (95% CI: 0.41, 0.70) times as likely to intend to use an unhygienic emptier the next time their on-site sanitation system needs emptying compared to controls (Table 4). A one-quartile increase in household wealth was associated with a decreased stated preference for future hygienic emptying at intervention compounds (aRR: 0.93, 95% CI: 0.88, 0.99) but we found no apparent association with control compounds (aRR: 0.99, 95% CI: 0.85, 1.15).

Among respondents with a stated preference for future unhygienic emptying, most cited cost as the reason they would not choose hygienic empty. Among the 34% (174/507) of control respondents who indicated they plan to unhygienically empty next time their sanitation system needs emptying, 91% (158/174) cited cost and 7% (12/174) cited access as their reason for not intending to use a hygienic emptier (Table A5). Similarly, among the 18% (100/542) of intervention respondents who indicated they plan to unhygienically

empty next time their sanitation system needs emptying, 81% (81/100) cited cost and 17% (17/100) cited access for not intending to use a hygienic emptier (Table A5). Intervention and control respondents who cited cost as their reason for not intending to hygienically empty had similar poverty scores (mean = 33/81, median = 31/81) compared to respondents who did not cite cost (mean = 30/81, median = 28/81).

Table 5. Response to stated preference survey questions

	All Survey Respondents			Household Respondents		Compound Leader	
	Control (n)	Intervention (n)	aRR (95% CI)	Control (n)	Intervention (n)	Control (n)	Intervention (n)
Respondent intends to use a hygienic emptier next time their on-site sanitation system needs emptying	18% (89/507)	58% (315/542)	3.1 (2.4, 4.0)	17% (63/378)	54% (216/399)	20% (26/129)	69% (99/143)
Respondent intends to use an intervention pit-emptier (from component two) next time their on-site sanitation system needs emptying	0.39% (2/507)	5.9% (32/542)	12 (2.8, 48)	0.53% (2/378)	5.3% (21/399)	0% (0/129)	7.7% (11/143)
Respondent intends to use an unhygienic emptier next time their on-site sanitation system needs emptying	34% (174/507)	18% (100/542)	0.54 (0.41, 0.70)	33% (123/378)	19% (75/399)	40% (51/129)	17% (25/143)

4.5 DISCUSSION

As cities in LMICs continue to grow in population and density, there is an increasing need for safe and sustainable sequestration of human fecal waste and hygienic FSM [9]. We assessed the impact of an intervention providing latrines with septic tanks and FSM services 24-36 months after delivery. Though the sample size for intervention compounds which reported emptying in the previous year was small, we found that the intervention was significantly associated with increased hygienic emptying of septic tanks, increased transportation of sludge to a WWTP or away from the living environment and increased stated intention to engage hygienic FSM services in the future.

The less frequent emptying observed at intervention compounds may have been due to the recent construction of the intervention and the size of the septic tanks which may require less frequent desludging than pit latrines in Maputo [74]. The proportion of shared latrines to community sanitation blocks that emptied in the previous year was the same as their proportion overall, suggesting both filled at similar rates. Intervention sanitation facilities were built during 2015-2016 and interviews took place in 2018. As some control respondents moved into their compounds after the on-site sanitation system had been constructed, we were unable to accurately determine the year that control systems were built for comparison. Additionally, intervention systems were not built in areas with high water tables; most control compounds were in the same neighborhoods as intervention compounds, but water table level was not a factor in control compound selection [78]. Infiltration of water from a high water table into control systems—which predominantly occurs during the rainy season in Maputo [87]—may have contributed to the observed increase in emptying frequency.

Fecal sludge in pour-flush systems is more watery than dry pit latrines and therefore more conducive to mechanized emptying than pit latrines which can be covered over or require manual removal of thick sludge [75]. As expected from pour flush systems, most hygienic emptying occurred at intervention compounds and control compounds with pour-flush sanitation technology; transport of fecal wastes to the WWTP or away from the compound only occurred following mechanized emptying. Additionally, a septic tank is a larger capital investment in the sub-surface infrastructure than a dry pit latrine, which incentivizes emptying over replacement. In unplanned settlements globally—where fecal sludge burial is increasingly unworkable—this suggests that upgrading pit latrines to pour-flush to septic tank systems, in the presence of affordable hygienic emptying, may be one way to increase the likelihood fecal wastes are safely managed. Though when space is available covering over old pits is an acceptable solution for safe management.

Our results suggest hygienic pit emptying remains unaffordable for many in Maputo's low-income neighborhoods; participants most often used cost to justify a stated preference for unhygienic emptying. Depending on the volume of fecal sludge removed and distance transported to the WWTP, hygienic mechanized emptying typically costs $25-$50 USD while unhygienic manually emptying costs $8-$17 USD (2015 data) [87]; wages in Mozambique may be $60 a month or less (2017 data) [168,169]. Household wealth was not associated with previous hygienic emptying which may suggest that emptying prices were expensive for both poor and relatively wealthier households. Wealthier intervention respondents were less likely to state a preference for future hygienic emptying than poorer intervention respondents. This may suggest courtesy or hypothetical response bias from poorer respondents [170]. Alternatively, poorer intervention residents may have been less likely than relatively wealthier residents to know the higher costs

associated with hygienic emptying and subsequently stated an increased preference for hygienic emptying.

Progress is ongoing in Maputo to subsidize emptying for low-income residents. The Maputo Municipal Council (MMC) approved a sanitation tariff in December 2016 which would tax Maputo residents' water bills to generate money. Revenue generated may be used for sanitation improvements and to subsidize hygienic emptying for low-income residents [151] A similar approach has already been implemented in eThekwini, South Africa, which offers free pit-emptying to households using pit latrines every five years [171]. The proposed tariff and potential subsidy in Maputo may help achieve affordable hygienic emptying for low-income residents.

Overflowing septic systems may result in a direct human exposure to fecal sludge, an issue of public health concern. A study in Bhutan found most building owners intended to wait until their septic tank was overflowing to initiate emptying [172]. We found a lower stated intention to delay emptying. Despite a recommendation to empty every two years in order to avoid overflowing, few intervention compounds reported an intention to empty based on time in use. Control compounds with pour-flush systems most often reported they use a visual inspection of fecal levels to time their pit-emptying. Intervention designs used in the Maputo Sanitation project made visual inspection of feces level impossible without masonry tools. Affordable, scheduled emptying of pour-flush to septic tank systems may be necessary to reduce intermittent exposure risks due to overflowing systems, especially in low lying areas prone to flooding in the rainy season. Additionally, a design feature such as an access port could be added to pour flush to septic tank systems to enable visual inspection, however access ports should not be easily removed to ensure a barrier exists between fecal waste and the domestic environment.

Although our observations have been limited to just one setting, our results suggest that, in this and potentially in similar unplanned urban settlements, subsidized on-site sanitation interventions designed for hygienic emptying may increase the likelihood of hygienic emptying compared to compounds which do not receive the intervention, including compounds that construct their own pour-flush systems [173]. Compounds with pour-flush sanitation may not have built their system in a location accessible by hygienic emptying or of a design compatible with the equipment used in hygienic emptying. A 2015 study in Dar es Salaam, Tanzania found that poor design or site placement of sanitation systems often necessitated damage to or destruction of the sanitation system in order for emptying to occur [153]. Educating residents of and masons working in low-income unplanned settlements about suitable site placement and design of sanitation infrastructure for hygienic emptying would be useful but may not be realistic in many LMICs. Additionally, intervention compounds more frequently reported a visit from WSUP where hygienic emptying was discussed; increased knowledge of hygienic emptying practices and the local providers of such services may have contributed to the observed increase in hygienic emptying at intervention compounds.

The decreased frequency of emptying at intervention compounds we observed may not have contributed to the increased use of hygienic emptying; the urban poor often base decisions on cash flow, not lifetime cost [174]. The economic reality for the urban poor suggests a sanitation levy to subsidize emptying may be necessary for safe management of fecal wastes. Although, when excreta are adequately sequestered on-site, a reduced emptying frequency subsequently reduces opportunities for human exposure to fecal waste and for excreta to spread into the environment, though hygienic emptying still poses risks for environmental fecal contamination. Where physically permissible, upgrading of traditional and improved latrines to pour flush to septic tank

systems may reduce environmental fecal contamination and downstream exposure risks to enteric pathogens simply from less frequent emptying, though some data suggests septic tanks may require emptying more often than pit latrines [75]

We reviewed the data provided to us by the local municipality to assess the prevalence of emptying activities by the intervention equipped pit-emptiers. Between August 2017 and February 2018 (excluding December 2017), the pit-emptiers accounted for 0.95% (112/11,831) of all truck visits to dispose of fecal sludge at the Infulene WWTP. Amongst trucks originating from intervention neighborhoods, the pit-emptiers accounted for 4.2% (28/667) of truck visits to the WWTP. Between August 2017 and February 2018, 25% (28/112) of the truck visits to the WWTP by the intervention pit-emptiers originated in intervention neighborhoods, suggesting the companies served low-income residents in the project area and residents outside the project area. Similar work by WSUP in Bangladesh demonstrated that for subsidized emptying companies a 70/30 mix of high-income and commercial customers to customers in low-income neighborhoods provided sufficient profit to encourage participation [175]. To guarantee service to low-income neighborhoods, WSUP subsidized emptiers in Bangladesh can be fined if <30% of their customers do not live in low-income neighborhoods. Where the target market cannot support the full cost of emptying, and enough high-income residents exist to provide a cross-subsidy, a similar approach may be useful to provide hygienic emptying services in Maputo and other LMICs.

The population density of *Nhlamankulu* district is high; of the 11 neighborhoods in the *Nhlamankulu* District five have 15,000-20,000 inhabitants/km^2 and six exceed 20,000 inhabitants/km^2 [6]. In this densely populated setting some compound residents did not state a preference for future hygienic emptying due to reported limited physical access by hygienic emptiers, which is common in informal settlements globally [155]. Pour flush systems produce a

greater volume of fecal sludge that when emptied unhygienically, is more mobile than the sludge from dry pit latrines and may subsequently pose greater risks for environmental fecal contamination. For areas in low income settlements inaccessible to hygienic emptying, general slum upgrading—especially during the expansion of piped water which was associated with an increase in pour flush systems in Maputo [87]—is crucial to safely manage fecal waste. Our results corroborate this previous observation in Maputo; greater access to tap water inside a compound correlated with an increase prevalence of pour-flush sanitation. Additionally, at high population densities simplified sewerage may have a lower annualized cost per capita than on-site sanitation [176]. Piped sewers and wastewater treatment should remain long-term goals in such a setting.

4.5.1 Limitations

There are various limitations to the reliability and external validity of our results. The cross-sectional nature of our study can only identify associations, not causation. Respondents were asked to remember past events, which introduced the potential for recall bias. Survey questions included hypothetical questions about the future, which introduced the potential for hypothetical response bias [170]. Due to the nature of the intervention, study participants were not blinded to the intervention, which may have led to interviewer or courtesy bias. As the intervention was recently implemented, very few intervention compounds had previously emptied their on-site sanitation system, which led us to draw conclusions about emptying practices from a small subset of all intervention compounds. Additionally, our findings represent emptying in one area of Maputo and may not be applicable to other cities in LMICs.

4.5.2 Conclusion

FSM is a critical area of urban health and development with few existing data; demographic trends indicate the population living in low-income unplanned settlements is increasing. Our results indicate that the provision of on-site sanitation systems consisting of pour-flush latrines to septic tanks, where safe and affordable emptying services are available, increased the use of such services but were unaffordable for many users.

CHAPTER 5. ENTERIC PATHOGEN DETECTION IN FECAL SLUDGES FOR ENVIRONMENTAL SURVEILLANCE IN LOW-INCOME URBAN SETTINGS

5.1 ABSTRACT

It is increasingly common to use sewage for health surveillance: analysis of wastewater can detect metabolites, biomarkers, and other biological targets excreted by communities. The actionable health relevant data from sewage surveillance suggests it may be useful for tracking outbreaks of emerging pathogens such as for SARS-Cov-2, but existing approaches do not consider low- and middle-income settings where non-sewered sanitation systems predominate and the burden of disease is high. To assess the utility of fecal sludges – collected from pit latrines and septic tanks – in enteric pathogen surveillance, we collected 95 matched stool and sludge samples from compounds (household clusters sharing sanitation and domestic space) in a low-income, urban community in Maputo, Mozambique. We analyzed samples for 20 common enteric pathogens via multiplex qPCR. Among the 95 stools matched to fecal sludges, we detected the six most prevalent bacterial pathogens (EAEC, *Shigella*/EIES, ETEC, EPEC, STEC, *Salmonella*) and all three protozoan pathogens (*Giardia*, *Cryptosporidium parvum*, *Entamoeba histolytica*) in the same rank order in both matrices; we observed the same rank detection order among pit latrines and septic tanks. Our results suggest that sampling fecal sludges from on-site sanitation offers potential for localized pathogen surveillance in low-income settings where enteric pathogen prevalence is high, but further work is needed to characterize the fate and transport of pathogens in fecal sludges, standardize sampling and analysis methods, and compare pathogen signals in sludges with wastewater.

5.2 INTRODUCTION

Wastewater monitoring is an increasingly used approach that has the potential for community health surveillance; sewage has been shown to provide useful community-level information on biomarkers of illicit drug use[177], antimicrobial resistance[178,179], and chronic disease.[180] Sewage has yielded advanced warning of viral outbreaks[181,182] and was useful to estimate the number of persons infected with SARS-CoV-2 in a catchment area.[84] Wastewater has benefits as a composite sample of a population's feces compared to stool-based surveillance from individuals, and the low relative cost compared to clinical, isolate-based surveillance led to a call for global sewage surveillance.[79] Pathogen surveillance in low- and middle-income countries (LMIC), where the burden of enteric disease is high, has not been demonstrated outside of sewage monitoring efforts for poliovirus.[83]

In high-income urban areas where the burden of disease is low, wastewater can be used in health surveillance because most cities have sewer systems.[2,80] In low-income urban areas on-site sanitation systems are common and sewers often serve a small fraction of the population.[183,184] Where sewers are absent, using fecal sludges in surveillance for a range of enteric pathogens is a logical and analogous sample with similar benefits to sewage sampling but at a much finer scale. Fecal sludge collection is biologically non-invasive, logistically easier and cheaper to collect than stool, and scalable across settings. As pathogens in fecal sludges indicate previous exposures among those contributing waste[185], using fecal sludge surveillance to identify the rank order of pathogens circulating in a community may be useful to inform comprehensive packages of WASH interventions tailored to address the local exposure landscape.[57]

For wastewater surveillance, a 24-hour composite sample of influent at a wastewater treatment plant is a representative sample for a catchment area.[178] Representative sampling of fecal sludges has not been standardized, but like wastewater, composite samples or multiple samples may be

necessary.[186,187] Wastewater may also provide a more current snapshot of circulating pathogens than fecal sludges, which remain sequestered until the pit is emptied or covered. How the detection of pathogens varies temporally and spatially inside pit latrines and septic tanks – which could inform sampling methods – remains poorly understood.[188] In addition, dry pit latrines produce thicker sludge than septic tanks which produce distinct layers of scum, liquids, and solids[22], suggesting different protocols may needed for representative sampling.

Parallel application of sampling techniques from wastewater systems to on-site systems in LMICs requires initial testing and validation using fecal sludges, including comparison with community infection prevalence. Our study aim was to determine if the rank order of enteric pathogens in fecal sludge from shared on-site sanitation systems at compounds (clusters of multiple households sharing common outside space and sanitation) enrolled in the Maputo Sanitation (MapSan) trial[90] was the same as the rank order of enteric pathogens in stool samples from children and infants. The MapSan trial is a non-randomized, controlled trial to evaluate the impact of a shared sanitation intervention on enteric infection and other health outcomes in children (Appendix A.1 **Detailed description of the sanitation intervention**). These results are a proof of concept in assessing whether fecal sludges have potential as a surveillance tool for enteric pathogen transmission among children in low-income neighborhoods where on-site sanitation is common.

5.3 METHODS

Our study took place in intervention and control compounds of low-income neighborhoods of Maputo, Mozambique, 24-months after the implementation of the shared sanitation intervention.[78,90] We used convenience sampling to collect fecal sludge from 95 MapSan compounds within 10 days of stool collection of an enrolled child (n=95) (October 2017-April

2018).[78,90] Methods for stool collection were previously described elsewhere.[78] We obtained children's age and household socioeconomic characteristics from the MapSan 24-month survey dataset.[43]

We sampled fecal sludge from pit latrines and septic tanks. For sampling pit latrines, we adapted a Sludge Nabber (Nasco, Fort Atkinson, WI) with a plastic tubing cover and a 50 mL centrifuge tube (Figure B1, Appendix

B**.1 Methodology for fecal sludge collection**). For septic tanks, we used a modified Wheaton Sub-Surface Sampler I system (Fisher Scientific, Waltham, Massachusetts) with a plastic insert to hold a 50-mL centrifuge tube (Figure B2, Appendix

B**.1 Methodology for fecal sludge collection**). All fecal sludge samples were stored on ice for transport, aliquoted into 2-ml cryovials within 6 hours of collection and stored at -80^{o}C at the Mozambican National Institute of Health. All samples were shipped from Maputo, Mozambique, to Atlanta, USA on dry ice (-80^{o}C) with temperature monitoring for molecular analysis.

5.3.1 Sample processing

For total nucleic acid extraction from 100 mg of stools and fecal sludges (wet-weight), we followed a pre-treatment protocol validated for multiplex PCR (Appendix B.2 **Methodology for nucleic acid extraction from fecal sludges and stools**).[78,115,158] We proceeded with extraction

following the manufacturer's protocol for the QIAamp 96 Virus QIAcube HT Kit, which we automated on the QIAcube (Qiagen, Hilden, Germany). We included MS2 as an extraction control.

We tested all samples using a custom TAC (ThermoFisher Scientific, Waltham, MA) that tested for 20 enteric pathogens, including ten bacteria, five viruses, three protozoa and two soil-transmitted helminths (STH) (Appendix B.3 **Custom TaqMan Array Card (TAC)**, Table B1) in duplicate. We included a positive and negative control on each TAC (Appendix B.3 **Custom TaqMan Array Card (TAC)**). We visually compared exponential curves and multicomponent plots with the positive control plots to validate positive amplification. Samples that exhibited positive amplification in one or both duplicate wells before a quantification cycle (Cq) of 40 were considered positive.

5.3.2 Data analysis

5.3.2.1 Predictors of the number of pathogens in stools and fecal sludges

To understand what variables were associated with the number of pathogens in stools and sludges, we investigated how children's age, compound wealth, compound population, and the type of on-site sanitation predicted the number of pathogens in sludges and stools. Our response variables included the the total number of pathogenic bacteria (range: 0-10), viruses (0-5), protozoa (0-3), and STHs (0-2) in stools and fecal sludges. Exposure variables representing potential contributors to the number of pathogens in stools and sludges were a wealth score compared to a one-quartile increase in wealth score[166], compound population compared to a 10-person increase in compound population, the type of on-site sanitation system compared to a pit latrine, and specifically for stools we included a categorical variable for child's age (1-23, 24-47, or 48-82 months) compared to children 1-23 months old.

We fit generalized linear models (GLM, Poisson regression with log link) to calculate unadjusted and adjusted prevalence ratios (PR, aPR) (Appendix B.4 **Regression models**). For this cross-sectional study, we define a prevalence ratio as the number of pathogenic bacteria, viruses, protozoa, or STHs for an exposure variable compared to the reference. We fit models that included all exposure variables simultaneously for stools and sludges, did not include any additional confounders in stool models (Figure B3), and included a sample's $\log_{10}$ transformed fecal sludge solids content as an additional covariate in fecal sludge models (Figure B4). Recognizing our analysis used multiple models, we applied a false discovery rate correction across taxa.[189] We analyzed data in R version 4.0.0 (R Foundation for Statistical Computing, Vienna, Austria).

5.3.2.2 Comparison of matched stools and fecal sludges

We used the presence of individual pathogens to compare stools and sludges. For matched samples, we used the Jaccard similarity coefficient[190] (e.g. the intersection of detections over the union). For example, out of our 95 samples, if we were to detect pathogen X in both a stool and the matched fecal sludge sample 40 times, only in stool 10 times, only in sludge 30 times, and in neither sample 15 times, the Jaccard similarity coefficient is 0.50 (Equation 1). Therefore, a coefficient of 0 would indicate there were no shared detections in stools and matched sludges, a coefficient of 0.50 would indicate half of the detections were shared, and a coefficient of 1 would indicate that all detections were shared.

Equation 1: $J_X = 40 \div (40 + 10 + 30) = 0.50$

In addition, to compare the rank order of pathogen prevalence, we determined each pathogen's prevalence in the 95 stools and 95 sludges. We separated pathogens by taxa and by matrix, sorted

pathogens from highest to lowest prevalence, then compared stools and sludges to determine which pathogens were detected in the same rank order between the two matrices.

5.4 RESULTS

5.4.1 Positive and negative controls

We observed positive amplification for all assays using our positive controls (n = 32). We observed no amplification for any assay in any of our extraction controls (n=8) and no template controls (n=24) below a quantification cycle (Cq) of 40.

5.4.2 Pathogens detected in stools

We collected stools from 95 children who ranged in age from 1-82 months (median = 37 months, mean = 39 months, SD = 21 months). In stools, we most often detected pathogenic bacteria (96%, [91/95]), followed by protozoa (68%, [65/95]), STHs (53%, [50/95]) and viruses (28%, [27/95]) (Table 6). The number of pathogens we detected per stool was high (mean=3.9 out of 20, median=4.0, range=0-9). Compared to the youngest children (1-23 months), we found stools from the oldest children (48-82 months) had no difference in the number of pathogenic bacteria (aPR = 1.3, 95% CI [0.87, 1.9]) or protozoa (aPR = 1.3 [0.66, 2.6]), but observed a lower number of viruses (aPR = 0.17 [0.05, 0.57]), and a greater number of helminths (aPR = 4.3 [1.8, 10]) (Table 7).

Table 6. Summary of pathogen prevalence (presence/absence) and mean number detected per taxa in stools and sludges

# of pathogens on TAC		Prevalence (95% CI)		Mean number detected (95% CI)	
		Stool (n=95)	Sludge (n=95)	Stool (n=95)	Sludge (n=95)
10	Bacteria	96% (92%, 100%)	95% (90%, 99%)	2.1 (1.9, 2.3)	2.9 (2.6, 3.2)
5	Viruses	28% (19%, 38%)	91% (95%, 96%)	0.36 (0.23, 0.49)	2.2 (2.0, 2.5)
3	Protozoa	68% (59%, 78%)	88% (82%, 95%)	0.77 (0.65, 0.89)	1.2 (1.1, 1.4)

2	STHs	53% (43%, 63%)	95% (90%, 99%)	0.74 (0.58, 0.90)	1.5 (1.4, 1.7)
20	All	99% (97%, 100%)	99% (97%, 100%)	3.9 (3.6, 4.3)	7.9 (7.4, 8.4)

5.4.3 Pathogens detected in fecal sludges

We collected 52 fecal sludge samples from septic tanks and 43 from pit latrines. The mean number of people per compound was 15 (SD=7, median=13, range=4-38). In fecal sludge we commonly detected all types of pathogens (bacteria: 95%, [90/95], STHs: 95%, [90/95], viruses: 91%, [86/95], protozoa: 88%, [84/95]) and in high number (mean=7.9 out of 20, median=8.0, range=0-14). In addition, we found that septic tank systems were associated with a reduced number of bacterial pathogens (aPR = 0.66, 95% [0.50, 0.86]) compared to pit latrines (Table 7).

Table 7. Associations between variables and the number of detected pathogenic bacteria, viruses, protozoa, or STHs in stools and sludges

Stool									
		Pathogenic bacteria		Pathogenic viruses		Pathogenic protozoa		STHs	
Variable	Reference	PR (95% CI)	aPR* (95% CI)	PR (95% CI)	aPR* (95% CI)	PR (95% CI)	aPR* (95% CI)	PR (95% CI)	aPR* (95% CI)
Age (24-47 months)	1-23 months	1.2 (0.84, 1.8)	1.2 (0.82, 1.7)	0.39 (0.16, 0.90)	0.41 (0.17, 0.97)	1.8 (0.99, 3.4)	1.9 (1.0, 3.6)	**3.8 (1.5, 9.4)**	**4.2 (1.7, 10)**
Age (48-82 months)		1.3 (0.87, 1.9)	1.2 (0.82, 1.7)	**0.17 (0.05, 0.57)**	**0.16 (0.05, 0.54)**	1.2 (0.63, 2.4)	1.3 (0.66, 2.6)	**4.3 (1.8, 11)**	**4.3 (1.8, 10)**
Wealth score	1-quartile increase	0.94 (0.83, 1.1)	0.95 (0.84, 1.1)	1.2 (0.89, 1.6)	1.1 (0.83, 1.5)	0.98 (0.80, 1.2)	0.98 (0.79, 1.2)	0.96 (0.78, 1.2)	0.98 (0.79, 1.2)
Compound population	10-person increase	1.1 (0.95, 1.4)	1.1 (0.93, 1.4)	0.83 (0.48, 1.3)	0.95 (0.53, 1.7)	1.1 (0.78, 1.5)	0.99 (0.71, 1.4)	0.99 (0.70, 1.4)	0.91 (0.64, 1.3)
On-site sanitation: pour-flush to septic tank system	Pit latrine	0.90 (0.68, 1.2)	0.86 (0.64, 1.2)	1.2 (0.60, 2.4)	1.1 (0.51, 2.2)	1.1 (0.71, 1.8)	1.1 (0.69, 1.8)	0.78 (0.49, 1.2)	0.89 (0.54, 1.4)
Sludge									
		Pathogenic bacteria		Pathogenic viruses		Pathogenic protozoa		STHs	
Variable	Reference	PR (95% CI)	aPR† (95% CI)	PR (95% CI)	aPR† (95% CI)	PR (95% CI)	aPR† (95% CI)	PR (95% CI)	aPR† (95% CI)
Wealth score	1-quartile increase	1.0 (0.91, 1.1)	1.0 (0.91, 1.1)	0.98 (0.87, 1.1)	1.0 (0.89, 1.1)	0.99 (0.85, 1.2)	1.0 (0.86, 1.2)	0.95 (0.83, 1.1)	0.96 (0.83, 1.1)
Compound population	10-person increase	1.0 (0.85, 1.2)	1.1 (0.94, 1.3)	**1.2 (1.0, 1.4)**	1.2 (0.97, 1.4)	1.1 (0.88, 1.5)	1.1 (0.85, 1.4)	0.97 (0.77, 1.2)	0.95 (0.75, 1.2)
On-site sanitation: pour flush to septic tank system	Pit latrine	**0.62 (0.49, 0.78)**	**0.66 (0.50, 0.86)**	**1.4 (1.1, 1.9)**	1.3 (0.96, 1.8)	1.2 (0.84, 1.8)	1.2 (0.78, 1.8)	1.1 (0.81, 1.6)	1.2 (0.85, 1.8)

Note: Bold indicates p≤0.05 following false discovery rate correction for multiple comparisons across taxa. PR: prevalence ratio. aPR: adjusted prevalence ratio. STH: soil transmitted helminth. CI: confidence interval.

*The full model was run for each taxa, which included children's age, wealth score, compound population, and sanitation system

†The full model was run for each taxa, which included wealth score, compound population, sanitation system, and the $\log_{10}$ transformed fecal sludge solids content

5.4.4 Stools and fecal sludges comparison

Among all stool and fecal sludge samples, we detected the six most frequent bacterial pathogens in stool in the same rank order of prevalence from fecal sludge (Table 8). We did not observe the same pattern for the viral pathogens, and the prevalence of all viruses was much greater in fecal sludge than in stool. We detected all three protozoan pathogens in the same order of prevalence in stool and fecal sludge. Additionally, we detected *Trichuris* more frequently than *Ascaris* in stool, but detected *Ascaris* more frequently than *Trichuris* in fecal sludge.

Table 8. Pathogens in stools and fecal sludges sorted by prevalence in stool (first column)

#	Pathogen	Stool (n = 95) (95% CI)	Sludge (n=95) (95% CI)	Jaccard similarity coefficient* (J)	Stool detections with a detection in the matched sludge (%)
	Bacteria				
1	EAEC	67% (58%, 77%)	82% (74%, 90%)	65% (56/96)	88% (56/64)
2	*Shigella*/EIES	51% (40%, 61%)	76% (67%, 84%)	45% (37/83)	77% (37/48)
3	ETEC (ST/LT)	38% (28%, 48%)	56% (46%, 66%)	33% (22/66)	63% (22/35)
4	EPEC	34% (24%, 43%)	39% (29%, 49%)	19% (11/57)	35% (11/31)
5	STEC (stx1/stx2)	6.3% (1.4%, 11%)	15% (7.6%, 22%)	0% (0/20)	0% (0/6)
6	*Salmonella*	6.3% (1.4%, 11%)	8.4% (2.8%, 14%)	0% (0/14)	0% (0/6)
7	Campylobacter *jejuni/coli*	5.3% (0.75%, 9.8%)	4.2% (0%, 8.3%)	0% (0/9)	0% (0/5)
8	*C. difficile* (tcdA/tcdB)	3.4% (0%, 6.7%)	7.4% (2.1%, 13%)	0% (0/10)	0% (0/3)
9	*Vibrio Cholerae*	0%	1.1% (0%, 3.1%)	0% (0/1)	
10	*Yersinia* spp.	0%	2.1% (0%, 5.0%)	0% (0/2)	
	Viruses				
1	Sapovirus I/II/IV/V	12% (5.1%, 18%)	47% (37%, 57%)	17% (8/48)	72% (8/11)
2	Norovirus GI/GII	11% (4.4%, 17%)	58% (48%, 68%)	8.3% (5/60)	50% (5/10)
3	Astrovirus	8.4% (2.8%, 14%)	63% (53%, 73%)	9.7% (6/62)	75% (6/8)
4	Adenovirus 40/41	4.2% (0%, 8.3%)	44% (34%, 54%)	7.0% (3/43)	75% (3/4)
5	Rotavirus A	1.1% (0%, 3.1%)	8.4% (2.8%, 14%)	13% (1/8)	100% (1/1)
	Protozoa				
1	*Giardia duodenalis*	64% (55%, 74%)	86% (79%, 93%)	63% (55/88)	90% (55/61)
2	*Cryptosporidium parvum*	12% (5.1%, 18%)	24% (16%, 33%)	9.7% (3/31)	27% (3/11)
3	*Entamoeba histolytica*	1.1% (0%, 3.1%)	12% (5.1%, 18%)	9.1% (1/11)	100% (1/1)
	STHs				
1	*Trichuris trichiuria*	42% (32%, 52%)	65% (56%, 75%)	42% (30/72)	75% (30/40)
2	*Ascaris lumbricoides*	32% (22%, 41%)	88% (82%, 95%)	31% (27/87)	90% (27/30)

*Size of the intersection of matched detections divided by the size of the union of detections. For example, we detected *Giardia* in both stool and the matched fecal sludge sample 55 times, only in stool 6 times, only in fecal sludge 27 times, and did not detect *Giardia* in either sample 7 times. E.g. $J_{Giardia} = (55) / (55 + 6 + 27) = 0.63$. Note: STH: soil-transmitted helminth. BL: baseline. 24M: 24-month. EAEC: *Enteroaggregative E. coli*. EIES: *Enteroinvasive E. coli*. ETEC: *Entertoxigenic E. coli*. EPEC: *Enteropathogenic E. coli*. STEC: shiga-toxin producing *E. coli*.

Dividing our analysis into compounds with pit latrines and septic tanks, we observed a similar trend. We detected the six most frequent bacterial pathogens in the same rank order from stools matched to pit latrines (EAEC, *Shigella*/EIES, ETEC, EPEC, STEC, and *Clostridium difficile*) and septic tanks (EAEC, *Shigella*/EIES, ETEC, EPEC, *Salmonella*, and *Campylobacter*) (Table 9). In addition for pit latrines and septic tanks we observed all three protozoan pathogens in the same rank order, STHs in opposite order, and the most prevalent viral pathogen was the same in stools matched to pit latrines (norovirus GI/GII) and septic tanks (astrovirus).

Table 9. Pathogen detection data disaggregated by sanitation infrastructure (septic tanks and latrines)

	Septic tanks			**Pit Latrines (with or without a slab)**		
	Stool (n = 52) (95% confidence interval)	Sludge (n = 52) (95% confidence interval)	Jaccard similarity coefficient (intersection / union)	Stool (n=43) (95% confidence interval)	Sludge (n=43) (95% confidence interval)	Jaccard similarity coefficient (intersection / union)
Bacteria						
EAEC	**62% (48%, 75%)**	**71% (59%, 84%)**	57% (25/44)	**74% (61%, 88%)**	**95% (89%, 100%)**	74% (31/42)
Shigella/EIES	**46% (32%, 60%)**	**62% (48%, 75%)**	37% (15/41)	**56% (41%, 71%)**	**93% (85%, 100%)**	52% (22/42)
ETEC (ST/LT)	**37% (23%, 50%)**	**44% (31%, 58%)**	35% (11/31)	**37% (23%, 52%)**	**70% (56%, 84%)**	31% (11/35)
EPEC	**33% (20%, 46%)**	**25% (13%, 37%)**	11% (3/27)	**33% (18%, 47%)**	**56% (41%, 71%)**	27% (8/30)
Salmonella	**9.6% (1.5%, 18%)**	**9.6% (1.5%, 18%)**	0% (0/10)	2.3% (0%, 6.9%)	7.0% (0%, 15%)	0% (0/4)
Campylobacter *jejuni/coli*	**5.8% (0%, 12%)**	**3.8% (0%, 9.1%)**	0% (0/5)	4.7% (0%, 11%)	4.7% (0%, 11%)	0% (0/4)
STEC (stx1/stx2)	3.8% (0%, 9.1%)	1.9% (0%, 5.7%)	0% (0/3)	**9.3% (0.52%, 18%)**	**30% (16%, 44%)**	0% (0/17)
C. difficile (tcdA/tcdB)	1.9% (0%, 5.7%)	3.8% (0%, 9.1%)	0% (0/3)	**4.7% (0%, 11%)**	**12% (1.9%, 21%)**	0% (0/7)
Vibrio Cholerae	0%	1.9% (0%, 5.7%)	0% (0/1)	0%	0% (0%, 0%)	NA
Yersinia spp.	0%	3.8% (0%, 9.1%)	0% (0/2)	0%	0% (0%, 0%)	NA
Viruses						
Sapovirus I/II/IV/V	**13% (4.1%, 23%)**	**52% (38%, 66%)**	13% (4/30)	9.3% (0.52%, 18%)	42% (27%, 57%)	22% (4/18)
Astrovirus	9.6% (1.5%, 18%)	75% (63%, 87%)	10% (4/40)	7.0% (0%, 15%)	49% (34%, 64%)	9.1% (2/22)
Norovirus GI/GII	9.6% (1.5%, 18%)	62% (48%, 75%)	8.8% (3/34)	**12% (1.9%, 21%)**	**53% (38%, 69%)**	7.7% (2/26)
Adenovirus 40/41	3.8% (0%, 9.1%)	56% (42%, 69%)	6.9% (2/29)	4.7% (0%, 11%)	30% (16%, 44%)	7.1% (1/14)
Rotavirus A	1.9% (0%, 5.7%)	12% (2.8%, 20%)	17% (1/6)	0%	4.7% (0%, 11%)	0% (0/2)
Protozoa						
Giardia duodenalis	65% (52%, 78%)	89% (82%, 98%)	62% (31/50)	63% (48%, 77%)	81% (70%, 93%)	63% (24/38)
Cryptosporidium parvum	13% (4.1%, 23%)	33% (20%, 46%)	9.1% (2/22)	9.3% (0.52%, 18%)	14% (3.5%, 24%)	11% (1/9)
Entamoeba histolytica	1.9% (0%, 5.7%)	9.6% (1.5%, 18%)	20% (1/5)	0% (0%, 0%)	14% (3.5%, 24%)	0% (0/6)
STHs						
Trichuris trichiuria	37% (23%, 50%)	71% (59%, 84%)	44% (17/39)	49% (34%, 64%)	58% (43%, 73%)	39% (13/33)
Ascaris lumbricoides	29% (16%, 41%)	90% (82%, 98%)	29% (14/48)	35% (20%, 49%)	86% (76%, 97%)	33% (13/39)

Note: bold indicates same rank order of detection in stools and sludges. STH: soil-transmitted helminth. BL: baseline. 24M: 24-month. EAEC: *Enteroaggregative E. coli*. EIES: *Enteroinvasive E. coli*. ETEC: *Entertoxigenic E. coli*. EPEC: *Enteropathogenic E. coli*. STEC: shiga-toxin producing *E. coli*.

5.4.5 Intra-compound stool and fecal sludge comparison

We detected every pathogen except *Campylobacter* more frequently in fecal sludge than in stool. Because we detected pathogens more frequently in sludges than in stools, the Jaccard similarity coefficients were highest among pathogens with the greatest prevalence in stool, lowest for pathogens with lowest prevalence in stools, and were zero for all bacterial pathogens detected in less than 10% of stools (Table 8). For bacteria, protozoa, and STHs with greater than 15% prevalence in stool, the pathogens detected in stools often had matched detections in fecal sludges; all viruses detected in stools were detected in the matched fecal sludges at least half the time (Table 8).

5.5 DISCUSSION

The same rank order of bacterial and protozoan pathogens in fecal sludges from pit latrines and septic tanks compared with pathogens in stools provides early evidence that fecal sludges may be a reliable endpoint for enteric pathogen surveillance in similar low-income urban settings. However, the Jaccard similarity coefficients for 18 of the 20 pathogens assessed were less than 0.5, indicating infrequent co-detection of pathogens in matched stools and sludges. Accordingly, surveillance of on-site sanitation systems may better provide a community level snapshot of circulating pathogens than serve as a reliable predictor of individual children's infections. Variations in the detected order and prevalence between stools and sludges – especially among viral targets – suggests additional work is needed to interpret pathogen detection based on shedding rates in stool[191,192], fate and transport[188,192,193], biological and environmental factors[188,192,193], assay limits of detection, and potential differences in nucleic acid extraction kit efficiencies.

Of the 20 pathogens assessed, the number of detections in fecal sludges (7.9) was twice that of stools (3.9), suggesting the fecal sludge samples represented a composite of multiple individuals' feces. On-site sanitation systems in this setting are commonly shared among households and had on average 15 residents potentially contributing waste. We analyzed one child's stool per compound – while sludges contained stools from all ages – which may explain why we did not observe high Jaccard similarity coefficients between pathogens detected in fecal sludges and stools.

Environmental persistence, shedding rates, and differences in infection prevalence with age may explain why we detected bacterial and protozoan pathogens from fecal sludge in the same rank order to children's stool, but not viruses and STHs. *Ascaris* can persist longer in the environment than *Trichuris*[192] but mass drug administration to treat helminthiasis is less effective for *Trichuris* than *Ascaris*.[194] Children enrolled in the MapSan trial received single-dose albendazole before the 24-month follow-up period, which may explain why we detected *Trichuris* more often in stools but *Ascaris* more often in sludges. We detected bacterial pathogens in the same rank order in stools and sludges, but bacteria may be subject to different environmental dynamics in other settings, such as die-off and re-growth[195], which could impact the rank order of bacterial pathogen detection. During viral gastroenteritis viruses are shed in high concentration (up to 10^{10}-10^{12} per gram feces)[191,192] and viral infections were associated with symptomatic diarrhea in this setting.[11] Watery diarrheal stools with a high concentration of viral particles may result in greater spatial distribution inside a latrine compared to hard lumpy stools, and may explain why we often detected viruses in sludges despite a relatively lower prevalence in stools. As expected from a previous study in this setting[78], we observed that children's age was associated with a reduced number of pathogenic viruses, and a greater number of pathogenic protozoa (though not after correcting for

multiple comparisons) and STHs, but not with bacteria. As children age their mobility and consumption of food and drinking water increases, which may increase infection risks. However, as children begin walking on their own, their direct contact with other people may decrease, which may lower their infection risk by viruses which can be spread via person-to-person transmission[196], and children may develop immunity against re-infection by viruses.[197] Considering that sludges are a composite from individuals with a wider range of ages than the stools we measured, it is logical that the rank order of viruses and STHs in sludges would not align with the rank order of stools from young children. In addition, the difference in the prevalence of the three protozoa in stools was large and may have limited the potential for change in the rank order detection from stool to sludge.

As a cross-sectional study we were unable to assess the sensitivity of pathogen signals to changes in disease prevalence or incidence over time. Longitudinal studies of fecal sludges in LMICs would be needed to assess these changes, including extension to early warning of disease outbreaks and to assess if fecal waste streams may be useful for health impact assessment in water, sanitation, and hygiene (WASH) intervention trials. We observed the same rank order prevalence for bacterial and protozoan pathogens from both pit latrines and septic tanks, except we detected fewer bacterial pathogens in septic tank sludges, and the six most prevalent bacterial pathogens were different between the two systems. Stratification of data by on-site sanitation infrastructure may provide helpful nuance to circulating pathogens. Unlike sewage which becomes increasingly homogenized as it flows from toilets to a treatment plant and offers a near real-time picture of community infections, sampling from on-site systems may be a more historical snapshot of previous infections. Given that we collected one sample per sanitation system in a 50 mL centrifuge tube at a single point just below the surface of the solids, it is plausible the sludges we collected represented older

feces than those at the solids surface. More work is needed to standardize sample collection techniques, which may include collecting and homogenizing a larger volume of sludge from each system.

In addition, we used the presence of pathogens, but quantitative work is needed to assess differences in concentrations and reconcile differences in assay limits of detection between sludges and stools. Molecular and viable pathogen concentrations in sludges would be helpful for risk assessment modeling, and to assess the relationship between concentration and the number of infected individuals contributing waste. In addition, compared to PCR, metagenomics offers an unbiased approach to assess the microbial community in on-site sanitation systems, and may be desired for comparison with global wastewater surveillance efforts.[79,178]

The ability to capture the relative frequency of enteric pathogens in a community – without the logistical constraints of stool collection, or the requirement for human subjects research scrutiny that applies in taking biological samples from individuals – offers the opportunity to rapidly gather novel information regarding community health. For urban settings with few resources, surveillance of fecal sludges may be a cheap and scalable option to monitor the spread emerging pathogens such as SARS-Cov-2.[80]Applying the principles of wastewater-based surveillance to areas covered by on-site systems is promising, but future work is needed to standardize methods and better characterize observed pathogen signals. Advances in surveillance where data is limited may help develop appropriate and effective strategies to control risk.

CHAPTER 6. IMPACT OF AN URBAN SANITATION INTERVENTION ON ENTERIC PATHOGENS IN SOILS

6.1 ABSTRACT

Environmental fecal contamination is ubiquitous in many low-income cities, which contributes to a high burden of enteric infections and associated poor health outcomes. To evaluate the impact of a shared on-site sanitation intervention on environmental fecal contamination we collected 179 latrine entrance soils – a standardized location in the domestic environment – from intervention (n=88) and control (n=91) compounds during the baseline and 24-month phase of the Maputo Sanitation Trial. We tested soils for the presence of 10 pathogenic bacteria, 5 pathogenic viruses, 3 pathogenic protozoa, and 2 soil-transmitted helminths (STH) using a multiplex PCR assay. Using a difference-in-difference (DID) analysis and adjusting for compound population, visibly wet soil, wealth, temperature, chickens/ducks, dogs, cats, and visible feces, we found evidence that the intervention reduced bacterial pathogen prevalence (adjusted prevalence ratio, aPR = 0.67, 95% CI: 0.46, 0.99) and number (aPR = 0.58, [0.34, 0.98]) in soils 24-months following the intervention, but had no effect on the prevalence or number of pathogenic viruses, protozoa, and STHs. We complemented our DID approach by assessing predictors for pathogen number in soils. Compared to pit latrines without slabs, intervention pour-flush sanitation systems were associated with a reduced number of bacterial pathogens (aPR = 0.57, [0.39, 0.82), viruses (aPR = 0.62, [0.37, 1.0]), and STHs (aPR = 0.56, [0.38, 0.83]) in soils. Results suggest the intervention may have reduced the spread of some fecal contamination into the domestic environment, but pathogens remained widely prevalent in latrine entrance soils 24-months following the intervention.

6.2 INTRODUCTION

On-site sanitation systems are designed to sequester human feces away from human contact and may therefore prevent the spread of fecal-oral pathogens through well-understood pathways.[23] Large-scale, rigorous randomized controlled trials (RCTs) of on-site sanitation systems – including sanitation alone and combinations of water, sanitation, and hygiene (WASH) interventions – found mixed effects on children's health.[38–40,42,44,198] Assessing the impact of WASH interventions on environmental fecal contamination is useful to understand the spread of enteric pathogens along the causal pathway from exposure to feces to new infection. For reasons of cost, capacity and feasibility, health impact studies often used fecal indicator bacteria (FIB) – a proxy for enteric pathogens – to assess environmental fecal contamination.[52,96,97,199,200] A 2016 meta-analysis, of studies that almost exclusively measured FIB, found improved sanitation had no effect on the spread of fecal contamination into the environment, possibly because fecal contamination is often pervasive in low-income environments[29,94,97,98,201,202] and some FIB may be naturalized in soils[55,56]. Measuring enteric pathogens, and not primarily commensal FIB, via PCR assays is increasingly used and offers improved insight into the impact of WASH interventions on the spread of such pathogens through the environment.[54,100,203,204]

There is a growing body of literature that soils contaminated by feces in public and domestic environments pose infection risks.[100,103,111,205] In health impact trials that assess improved on-site sanitations systems, latrine entrance soils are assessed to measure how effectively the intervention sequestered human feces.[94,98,206] Indeed, latrines and septic tanks are useful barriers against the spread of feces, but enteric pathogens may still spread into latrine entrance soils through open defecation, unhygienic pit emptying, improper disposal of children's feces or anal cleansing materials, latrine flooding, and from animal feces.[5,78,146,207] Domestic soils contaminated by enteric pathogens can pose infection risks beyond incidental[208] and direct[102] soil ingestion; contaminated

soil may spread to hands, food, fomites, or household stored water.[97] For these reasons, latrine entrance soils may be a useful matrix to assess the impact of on-site sanitation interventions on soil contamination.

The Maputo Sanitation (MapSan) Trial was the first controlled trial to rigorously evaluate the effect of an urban on-site sanitation intervention on children's health outcomes. We conducted the trial in low-income, informal neighborhoods in Maputo, Mozambique, where WASH conditions are poor and the burden of enteric disease is high.[29,78,207] Water and Sanitation for the Urban Poor (WSUP, a non-governmental organization) delivered the intervention at compounds (household clusters who shared sanitation and courtyard space). WSUP replaced shared on-site sanitation systems in poor condition with pour-flush toilets that included septic tanks and soak-away pits (Appendix A.1). Control compounds were concurrently enrolled from the same or adjacent neighborhoods to intervention compounds and continued using existing shared sanitation infrastructure. Detailed descriptions of the intervention and inclusion criteria for intervention and control compounds is described elsewhere.[78,207]

We assumed that latrine entrance soils located in the domestic environment – which we defined as the location one-meter away from the latrine entrance in the direction of entry or the nearest point not covered by cement – may receive a consistent input of fecal material from on-site sanitation systems that poorly sequester fecal waste. Due to their close proximity to contained feces, latrine entrance soils may be at a greater risk of contamination than soil from other locations inside the domestic environment, but are where we expected to observe the greatest reduction in enteric pathogens if the intervention infrastructure performed better than controls at containing fecal wastes. Soils in low-income Maputo are characterized as coarse to fine sand or silty sand.[209] While the fate and transport of pathogens through soils is dependent on a pathogen's biology and

environmental conditions[210], the large pores in sandy soils enable greater movement of pathogens[211], further suggesting latrine entrance soils in this setting may be at risk of fecal contamination from poorly functioning sanitation systems.

Our study aims were (1) to assess if the intervention reduced the prevalence or number of pathogenic bacteria, viruses, protozoa, or soil-transmitted helminths (STH) in latrine entrance soils from MapSan intervention compounds compared to controls, and (2) to identify predictors of the number of pathogenic bacteria, viruses, protozoa, or STHs in latrine entrance soils in low-income urban Maputo, Mozambique. We anticipated the predictor analysis would provide supplementary evidence for the mechanisms that may spread fecal contamination into latrine entrance soils.

6.3 METHODS

6.3.1 Sample Collection

We prospectively collected latrine entrance soil samples from 50 intervention and 50 control compounds at baseline (before the intervention) and from the same compounds 24-months following the intervention, for a total of 200 samples. Using a spade and ruler (sterilized between uses) we scooped a 10 cm x 10 cm x 1 cm volume of soil into a whirl-pak bag (Nasco, Fort Atkinson, WI). At the time of sampling enumerators recorded whether the soil was visibly wet. Samples were stored on ice for transport, frozen at -20^{o}C for approximately 6 months, aliquoted into 2-ml cryovials while working on dry ice, and then stored at -80^{o}C until analysis. During storage, some samples were lost as the permanent marker labeling on some whirl-pak bags wore off and some bags burst open. All aliquoted samples were shipped from the Mozambican Ministry of Health in Maputo, Mozambique to Atlanta, GA, USA on dry ice (-80° C) with temperature

monitoring for molecular analysis. We obtained compound observation data and socioeconomic characteristics from the MapSan baseline and 24-month survey datasets.[43,78]

6.3.2 Sample Processing

We incubated 250 mg of each soil sample at 105°C for 1 hour to determine moisture content[212], discarded the dry soil, then extracted total nucleic acids from a separate 1-gram portion of each sample (dry weight), and spiked samples with MS2 as an extraction control. Following the manufacturer's protocol, we extracted RNA using the RNeasy PowerSoil Total RNA Kit and DNA using the RNeasy PowerSoil DNA Elution Kit (Qiagen, Hilden, Germany). On each day of extractions (5-15 samples), we included one negative extraction control. We tested soil samples for inhibition using the Applied Biosystems Exogenous Internal Positive Control Assay[213] (Applied Biosystems, Waltham, Massachusetts) before downstream molecular analysis.

We tested all samples using a custom TaqMan Array Card (TAC) (ThermoFisher Scientific, Waltham, MA) that tested for 20 enteric pathogens, including ten bacteria (*Campylobacter jejuni/coli*, *Clostridium difficile* [*tcdA* and *tcdB* gene], *Enteroaggregative E. coli* [EAEC, *aaiC* and *aatA* gene], *Shigella/Enteroinvasive E. coli* [EIEC, *ipaH* gene], *Enteropathogenic E. coli* [EPEC, *bfpA* and *eae* gene), *Enterotoxigenic E. coli* [ETEC, heat-labile and heat-stabile enterotoxin gene], shiga-toxin producing *E. coli* [STEC, *stx1* and *stx2*], *Salmonella* spp., *Vibrio cholerae*, and *Yersinia* spp.), five viruses (Adenovirus 40/41, Astrovirus, Norovirus [GI and GII], Rotavirus A, and Sapovirus [I, II, IV, and V],), three protozoa (*Cryptosporidium parvum*, *Entamoeba histolytica*, and *Giardia duodenalis*,) and two STHs (*Ascaris lumbricoides*, *Trichuris trichiuria*) in duplicate (Table B1). We combined and then added 25 µL of RNA eluant, 25 µL of DNA eluant, and 50 µL of mastermix into each TAC port. We included a positive and negative control on each

TAC. The positive control was a plasmid that included all assay gene sequences. The thermocycling conditions were as follows: 45°C for 10 minutes and 94°C for 10 minutes, followed by 45 cycles of 94°C for 30 seconds and 60°C for 1 minutes, with a ramp rate of 1°C/second between each step. We visually compared exponential curves and multicomponent plots with the positive control plots to validate positive amplification[100]; positive amplification in one or both duplicate wells below a quantification cycle (Cq) of 40 was called as a positive for a target.

6.3.3 Data analysis

We analyzed data in R version 4.0.0 (R Foundation for Statistical Computing, Vienna, Austria). We applied the intention-to-treat principle and used a difference-in-difference (DID) approach to assess the impact of the intervention – our exposure variable – on our outcomes compared to the control group. Our outcomes included the prevalence (i.e. binary presence/absence) and the total number of unique pathogenic bacteria, viruses, protozoa, and helminths in latrine entrance soils. We stratified the number of pathogens by taxa because environmental persistence is most similar amongst pathogens within a taxa.[193] We used generalized estimating equations (GEE)[214] to fit unadjusted and adjusted Poisson regression models with robust standard errors, including an "exchangeable" correlation structure. We accounted for clustering between compounds across the two study phases because this was the level of intervention implementation.[215]

To generate adjusted estimates, we selected 8 covariates from the MapSan baseline and 24-month datasets based on their biological plausibility[29,97] to impact the spread or persistence of fecal contamination in the domestic environment. We used the same 8 covariates to adjust all difference-in-difference models, which included a 10-person increase in compound population, 1-quartile increase in wealth index[166], a binary variable for visibly wet soil at the time of sampling, the mean-

centered average air temperature in Fahrenheit for the day of and day preceding sample collection (i.e. 2-day average), a binary variable for the presence of cats, a binary variable for the presence of dogs, a binary variable for the presence of chickens or ducks, and a binary variable for the presence of visible animal or human feces in the compound (Table C1).

To estimate the effect of the intervention we used the interaction of dummy variables representing treatment status (intervention vs. control) and trial phase (baseline or 24-month). Consequently, we present the effect estimates from our DID analysis as ratio measures (ratio of prevalence ratios, PR) instead of absolute differences. We fit separate models to predict the presence and number of pathogenic bacteria, viruses, protozoa, and STHs. Likewise, we fit DID models to estimate the intervention's impact for each pathogenic bacteria, virus, protozoa and STH, but we excluded any pathogen not detected in at least 5% of combined control and intervention samples at the 24-month phase.

In addition, to account for the unplanned crossover of some compounds between study groups, we conducted a sensitivity analysis that excluded crossover compounds. We used the same DID approach as in our intention-to-treat analysis, aiming to assess if the inclusion of crossover compounds may have biased our DID estimates towards the null.[216]

To further evaluate predictors of the number of pathogenic bacteria, viruses, protozoa, or STHs detected in latrine entrance soils from both study phases, we used generalized estimating equations (GEE) to fit Poisson regression models with robust standard errors. We clustered the data by compounds, and due to the repeated cross-sectional nature of the study, we calculated unadjusted and adjusted prevalence ratios (PR, aPR). We investigated nine exposure variables representing potential sources of enteric pathogens in soils or factors contributing to pathogen persistence,

which included the on-site sanitation infrastructure, compound population, visibly wet soil, wealth index, 2-day average temperature, presence of chickens/ducks, presence of dogs, presence of cats, and visible feces in the compound. For this analysis, we define prevalence ratio as the prevalence of the detected number of pathogenic bacteria, viruses, protozoa, or STHs for an exposure variable compared to the reference: type of on-site sanitation system versus pit latrine without a slab, a 10-person increase in compound population, visibly wet soil versus visibly dry, 1-quartile increase in wealth index, 10^{o} F increase in 2-day average temperature, chicken(s)/duck(s) present versus none, dog(s) present versus none, cat(s) present versus none, and visible feces versus none. To generate adjusted estimates, we fit the full model, which included all nine variables. In addition, we assessed the association between full pit latrines and the number of pathogenic bacteria, viruses, protozoa, or STHs detected in latrine entrance soils compared to latrines that were not visibly full. However, we only recorded whether a pit was full at the 24-month phase and consequently did not include baseline data in the full pit regression models. To generate adjusted estimates for the full pit regression models we adjusted for the other nine variables assessed in our predictor analysis. Recognizing that our analysis aimed to perform multiple tests, we adjusted for multiple comparisons across taxa using a false discovery rate correction.[189]

6.3.4 Ethics

The study protocol was approved by the Comité Nacional de Bioética para a Saúde (CNBS), Ministério da Saúde (333/CNBS/14), the Research Ethics Committee of the London School of Hygiene and Tropical Medicine (reference # 8345), and the Institutional Review Board of the Georgia Institute of Technology (protocol # H15160). The overall trial was pre-registered at ClinicalTrials.gov (NCT02362932), but we did not pre-register this environmental analysis.

6.4 RESULTS

6.4.1 Matched samples

We analyzed latrine entrance soils collected at baseline from 47 control compounds and 44 intervention compounds, and soils collected at the 24-month phase from 44 control and 44 intervention compounds (Table 10). Among control soils, 42 were matched by compound from baseline to the 24-month period, with 5 unmatched soils at baseline and 2 unmatched at 24-months. Among intervention soils, 41 were matched by compound from baseline to the 24-month period, with 3 unmatched soils at baseline and 3 unmatched at 24-months. There was a mean of 781 days between the collection of matched control samples (sd = 34, min = 733, max = 858) and a mean of 796 days between matched intervention samples (sd = 57, min = 731, max = 953) (Figure C1).

Table 10. **Soils samples matched at baseline and 24-month trial periods**

Latrine entrance soil samples	**Control**	**Intervention**
Just baseline	5	3
Matched baseline and 24-month	42	41
Just 24-month	2	3
Total baseline	47	44
Total 24-month	44	44

6.4.2 Compound characteristics

Control and intervention compounds had similar wealth indexes at baseline and the 24-month phase (Table C3). In addition, at baseline there were more residents – but not significantly so – at intervention compounds (mean = 18, standard deviation = 7.6) compared to controls (mean = 15, standard deviation = 7.1. t-test p = 0.11), but compound populations were similar at the 24-month phase (Table C3). At baseline, control compounds more often had pit latrines with slabs (64%,

[30/47]) than without slabs (26%, [12/47]), compared to intervention compounds which more often had pit latrines without slabs (64%, [28/44]) than with slabs (25%, [11/44]). By the 24-month phase 23% (10/44) of control compounds had independently upgraded their pit latrines to pour-flush toilets, while 2.3% (1/44) had a pour-flush toilet at both baseline and the 24-month phase (Table C2). In addition, some crossover between study arms occurred. At the 24-month phase 14% (6/44) of control compounds had the intervention sanitation infrastructure present and 11% (5/44) of intervention compounds did not have the intervention infrastructure present (Table C2). We excluded the 24-month phase results of these crossover compounds from the sensitivity analysis.

6.4.3 Controls

We observed positive amplification for all assays using our positive controls (n = 32). We did not observe positive amplification for any assay in any of our extraction controls (n=16) or no template controls (n=16) below a quantification cycle (Cq) of 40.

6.4.4 Bacteria

We detected at least one pathogenic bacteria in 72% (128/179) of latrine entrance soils, co-detected two or more pathogenic bacteria in 40% (72/179) of samples, and detected a mean of 1.5 pathogenic bacteria out of 10 per sample (range 0-8). We found evidence the intervention reduced the prevalence of pathogenic bacteria overall (aPR = 0.67, 95% CI [0.46, 0.99]) and reduced the number of pathogenic bacteria (aPR = 0.58, [0.34, 0.97]) (Table 11).

Although it is possible that the observed effect estimates on individual bacteria prevalence may have occurred by chance, since the confidence intervals crossed one, there was a consistent trend among the six most prevalent bacterial pathogens (*Enteroaggregative E. coli* (EAEC),

Shigella/Enteroinvasive E. coli (EIES), *Enterotoxigenic E. coli* (ETEC), *Enteropathogenic E. coli* (EPEC), *Clostridium difficile*, and *Salmonella*); the point estimates for all six suggest the intervention had a protective effect (

Table **13**). In fact, we found evidence that the intervention reduced the prevalence of EPEC (PR = 0.23, [0.06, 0.92]) in latrine entrance soils, but the estimate had a wide confidence interval and was not significant in the adjusted estimate (aPR = 0.26 [0.06, 1.1]).

6.4.5 Viruses

We detected at least one pathogenic virus in 44% (79/179) of latrine entrance soils and co-detected two or more pathogenic viruses in 13% (23/179) of samples (range 0-3). At baseline and the 24-month phase we most often detected Astrovirus (26%, [47/179]) and Adenovirus 40/41 (20%, [35/179]), while Rotavirus (8.9%, [16/179]) and Norovirus GI/GII (4.5%, [8/179]) were detected less often, and we did not detect Sapovirus (Table 11). We observed no effect of the intervention on the prevalence of any pathogenic virus overall, the number of pathogenic viruses, or any individual pathogenic virus (Table 11,

Table **13**).

6.4.6 Protozoa

We detected at least one pathogenic protozoa in 41% (74/179) of latrine entrance soils and co-detected two pathogenic protozoa in 3.4% (6/179) samples. At baseline and the 24-month phase we most often detected *Giardia duodenalis* (36% [64/179]), followed by *Cryptosporidium parvum* (7.3% [13/179]), and *Entamoeba histolytica* (1.7%, [3/179]). We found no evidence the intervention had an effect on the prevalence of any pathogenic protozoa overall, the number of pathogenic protozoa, or any individual pathogenic protozoa (Table 11,

Table **13**).

6.4.7 STHs

We detected *Ascaris* or *Trichuris* in 64% (115/179) of latrine entrance soils and co-detected both in 21% (38/179) of samples. We detected *Ascaris* in most samples (62% [111/179]) and *Trichuris* in approximately one-quarter of samples (23% [42/179]). We found no evidence the intervention reduced the prevalence of any STH overall, the number of STHs, or any individual STH (Table 11,

Table **13**).

6.4.8 Sensitivity analysis

We performed a sensitivity analyses that excluded the 24-month data from the 14% (6/44) of control compounds that had the intervention sanitation infrastructure present and the 11% (5/44) of intervention compounds that did not have the intervention infrastructure present at the 24-month phase (Table C4). Similar to the intention-to-treat estimates, in the sensitivity analysis we observed the intervention reduced the prevalence (aPR = 0.66 [0.44, 0.98]) and number (aPR = 0.55, [0.31,0.94]) of bacterial pathogens, but had no effect on the prevalence or number of pathogenic viruses, protozoa, and STHs. Likewise, we observed a protective effect on individual bacterial pathogens, a significant reduction in EPEC (aPR = 0.20, [0.04, 0.87]). However, unlike the estimate from our intention-to-treat analysis, in the sensitivity analysis we observed the intervention was also protective against EAEC (aPR = 0.54, [0.29, 1.0]).

Table 11. **Total number and prevalence of pathogens at baseline and 24-month**

Prevalence					
	Baseline Prevalence	24-month Prevalence		Unadjusted BL-24M DID estimate	Adjusted BL-24M DID estimate
Any pathogenic bacteria					
control	0.64 (30/47)	0.77 (34/44)			
intervention	0.80 (35/44)	0.66 (29/44)		0.68 (0.45, 1.0)	**0.67 (0.46, 0.99)**
Any pathogenic virus					
control	0.51 (24/47)	0.48 (21/44)			
intervention	0.39 (17/44)	0.39 (17/44)		1.1 (0.56, 2.1)	1.1 (0.57, 2.2)
Any pathogenic protozoa					
control	0.47 (22/47)	0.36 (16/44)			
intervention	0.41 (18/44)	0.41 (18/44)		1.3 (0.66, 2.4)	1.5 (0.77, 2.9)
Any STH					
control	0.72 (34/47)	0.75 (33/44)			
intervention	0.61 (27/44)	0.48 (21/44)		0.75 (0.49, 1.2)	0.79 (0.50, 1.2)
Any pathogenic bacteria, virus, protozoa, or STH					
control	0.91 (43/47)	0.95 (42/44)			
intervention	0.93 (41/44)	0.86 (38/44)			

Table 12 continued

Number					
	Baseline: mean # detected, range	24-month: mean # detected, range		Unadjusted BL-24M DID estimate	Adjusted BL-24M DID estimate
Pathogenic bacteria (out of 10)					
control	1.5 (0, 5)	1.8 (0, 8)			
intervention	1.6 (0, 5)	1.1 (0, 4)		**0.57 (0.34, 0.98)**	**0.58 (0.34, 0.97)**
Pathogenic viruses (out of 5)					
control	0.70 (0, 3)	0.61 (0, 2)			
intervention	0.52 (0, 3)	0.52 (0, 3)		1.1 (0.55, 2.4)	1.2 (0.58, 2.6)
Pathogenic protozoa (out of 3)					
control	0.51 (0, 2)	0.39 (0, 2)			
intervention	0.45 (0, 2)	0.43 (0, 2)		1.2 (0.61, 2.5)	1.4 (0.67, 2.9)
STHs (out of 2)					
control	1.1 (0, 2)	0.93 (0, 2)			
intervention	0.80 (0, 2)	0.61 (0, 2)		0.89 (0.53, 1.5)	0.94 (0.56, 1.6)
Sum of pathogenic bacteria, viruses, protozoa, and STHs					
control	3.8 (0, 11)	3.8 (0, 13)			
intervention	3.3 (0, 8)	2.7 (0, 8)			

Note: bold indicates $p \leq 0.05$. DID: difference-in-difference. STH: soil transmitted helminth. BL: baseline. 24M: 24-month.

Table 13. **Prevalence of individual pathogens at baseline and 24-month. Sorted by prevalence in control soils at the 24-month phase.**

Pathogenic bacteria					
	Baseline Prevalence	24-month Prevalence		Unadjusted BL-24M DID estimate[ǂ]	Adjusted BL-24M DID estimate[ǂ]
EAEC (*aaiC*/*aatA*)					
control	0.43 (20/47)	0.5 (22/44)			
intervention	0.5 (22/44)	0.41 (18/44)		0.70 (0.38, 1.3)	0.63 (0.34, 1.1)
Shigella/EIES (*ipaH*)					
control	0.34 (16/47)	0.36 (16/44)			
intervention	0.16 (7/44)	0.07 (3/44)		0.40 (0.10, 1.6)	0.46 (0.12, 1.8)
ETEC (*ST*/*LT*)					
control	0.26 (12/47)	0.36 (16/44)			
intervention	0.36 (16/44)	0.23 (10/44)		0.54 (0.21, 1.4)	0.59 (0.23, 1.5)
EPEC (*bfpA*/*eae*)					
control	0.13 (6/47)	0.23 (10/44)			
intervention	0.23 (10/44)	0.09 (4/44)		**0.23 (0.06, 0.92)**	0.26 (0.06, 1.1)
C. difficile (*tcdA*/*tcdB*)					
control	0.11 (5/47)	0.16 (7/44)			
intervention	0.18 (8/44)	0.14 (6/44)		0.49 (0.12, 2.1)	0.47 (0.11, 2.1)
Salmonella					
control	0.02 (1/47)	0.11 (5/44)			
intervention	0.05 (2/44)	0.05 (2/44)		0.21 (0.01, 4.3)	0.10 (0.01, 1.4)
STEC (stx1/stx2)					
control	0.04 (2/47)	0.09 (4/44)			
intervention	0 (0/44)	0.02 (1/44)		NA	

Table 14 continued

Campylobacter *jejuni*/*coli*					
control	0.15 (7/47)	0.07 (3/44)			
intervention	0.09 (4/44)	0.07 (3/44)		1.6 (0.32, 7.8)	1.4 (0.22, 8.8)
Yersinia spp.					
	0.04 (2/47)	0.05 (2/44)			
	0.02 (1/44)	0.05 (2/44)		1.9 (0.09, 41)	3.1 (0.15, 65)
Vibrio Cholerae					
control	0 (0/47)	0 (0/38)			
intervention	0 (0/44)	0 (0/39)		NA	
Pathogenic viruses					
Astrovirus					
control	0.26 (12/47)	0.32 (14/44)			
intervention	0.2 (9/44)	0.32 (14/44)		1.3 (0.54, 3.3)	1.5 (0.56, 4.1)
Adenovirus 40/41					
control	0.26 (12/47)	0.3 (13/44)			
intervention	0.11 (5/44)	0.11 (5/44)		0.84 (0.22, 3.3)	0.80 (0.21, 3.0)
Norovirus (GI/GII)					
control	0.06 (3/47)	0.02 (1/44)			
intervention	0.07 (3/44)	0.02 (1/44)		NA	
Rotavirus A					
control	0.13 (6/47)	0.00 (0/44)			
intervention	0.14 (6/44)	0.09 (4/44)		NA	
Sapovirus (I/II/IV/V)					
control	0 (0/47)	0 (0/38)			
intervention	0 (0/44)	0 (0/39)		NA	

Table 15 continued

Pathogenic protozoa					
Giardia duodenalis					
control	0.43 (20/47)	0.34 (15/44)			
intervention	0.39 (17/44)	0.30 (13/44)		1.0 (0.46, 2.2)	1.2 (0.53, 2.5)
Cryptosporidium parvum					
control	0.06 (3/47)	0.07 (3/44)			
intervention	0.07 (3/44)	0.16 (7/44)		2.3 (0.24, 22)	2.1 (0.18, 25)
Entamoeba histolytica					
control	0.02 (1/47)	0 (0/44)			
intervention	0.02 (1/44)	0.02 (1/44)		NA	NA
STHs					
Ascaris lumbricoides					
control	0.68 (32/47)	0.75 (33/44)			
intervention	0.59 (26/44)	0.45 (20/44)		0.70 (0.45, 1.1)	0.72 (0.45, 1.1)
Trichuris trichiuria					
control	0.38 (18/47)	0.18 (8/44)			
intervention	0.2 (9/44)	0.16 (7/44)		1.6 (0.52, 5.2)	1.8 (0.55, 5.6)

Note: bold indicates p≤0.05. DID: difference-in-difference. STH: soil-transmitted helminth. BL: baseline. 24M: 24-month. EAEC: *Enteroaggregative E. coli*. EIES: *Enteroinvasive E. coli*. ETEC: *Entertoxigenic E. coli*. EPEC: *Enteropathogenic E. coli*. STEC: shiga-toxin producing *E. coli*.

ǂWe did not calculate DID estimates for pathogens with <5% prevalence at the 24-month phase or where no pathogen was detected in either arm at a single phase

6.4.9 Predictors of the number of pathogens in soils

6.4.9.1 Sanitation infrastructure

Including compounds from baseline and the 24-month phase, sanitation most often took the form of pit latrines with slabs (33%, [59/179]), followed by pit latrines without slabs (30%, [54/179], intervention pour-flush toilets (25%, [45/179]), non-intervention pour-flush toilets (8.4%, [15/179]), and resorting to open defecation or using a neighbor's latrine (3.9% [7/179]). In adjusted models that included data from both study phases, intervention pour-flush toilets were associated with a reduced number of pathogenic bacteria (aPR = 0.57, [0.39, 0.82]), pathogenic viruses (aPR = 0.62, [0.37, 1.0]), and STHs (aPR = 0.56, [0.38, 0.83]) compared to pit latrines without slabs, but were not associated with a reduced number of pathogenic protozoa (aPR = 0.78, [0.49, 1.3) (Table 16). Though, the association with pathogenic viruses was not significant after adjusting for multiple comparisons. Non-intervention pour-flush toilets were also associated with a reduced number of pathogenic viruses in latrine entrance soils (aPR = 0.22, [0.06, 0.81]), though not significantly after adjusting for multiple comparisons, and were not associated with the number of pathogenic bacteria, protozoa, or STHs. Compared to pit latrines without slabs, pit latrine with slabs were not associated with the number of pathogenic bacteria, viruses, protozoa, or STHs. In addition, for compounds who reported open defecation or using a neighbor's latrine – but had a latrine on premises to sample at – we found a reduced number of pathogenic bacteria in latrine entrance soils (aPR = 0.29 [0.15, 0.57]) compared to pit latrines without slabs. We observed no association between open defecation or using a neighbor's latrine and the number of pathogenic viruses, protozoa, or STHs in latrine entrance soils compared to pit latrines without slabs.

6.4.9.2 Compound population

There was a general trend that increasing compound population was associated with a greater number of pathogens in latrine entrance soils; we found a 10-person increase in compound population was associated with a greater number of pathogenic bacteria (aPR = 1.2, [1.0, 1.4]), pathogenic viruses (aPR = 1.3, [1.0, 1.6]), and pathogenic protozoa (aPR = 1.3, [1.0, 1.6]), and was marginally associated with a greater number of STHs (aPR = 1.1, [0.94, 1.3]). Though none of these associations were significant after adjusting for multiple comparisons.

6.4.9.3 Visibly wet soil

Across both study phases, we more often observed that soils were visibly wet (79%, [142/179]) than visibly dry (21%, [37/179]). Compared to visibly dry soil, there was no association between visibly wet soil and the number of pathogenic taxa in soils. However, the point estimates for pathogenic bacteria (aPR = 1.2, [0.85, 1.6]), viruses (aPR = 1.2, [0.76, 1.8]), protozoa (aPR = 1.3, [0.73, 2.3]), and STHs (aPR = 1.3 [0.93, 1.7]) were similar, which may suggest that visibly wet soil was associated with a greater number of pathogens compared to visibly dry soil.

6.4.9.4 Wealth index

In adjusted estimates we found no associations between a 1-quartile increase in wealth index and the number of pathogenic taxa in soils. Though, in an unadjusted estimate we observed a 1-quartile increase in wealth index was associated with a reduced number of pathogenic viruses in soils (PR = 0.87, [0.74, 1.0]), but this association was not significant after adjusting for multiple comparisons.

6.4.9.5 Two-day average temperature

The 2-day average temperature from the day of and day preceding each sample was 72^{o} F (standard deviation = 5, median = 70, minimum = 65, maximum = 84). In adjusted models, a 10^{o} F increase in temperature was not associated with the number of pathogenic taxa in soils. Though, we found similar point estimates for the number of pathogenic viruses (aPR = 0.86, [0.58, 1.3]), protozoa (aPR = 0.70, [0.43, 1.1]), and STHs (aPR = 0.83, [0.62, 1.1]). The homogeneity in these estimates may suggest higher temperatures had a protective effect against the number of pathogens in soils.

6.4.9.6 Animals

Most compounds often possessed cats (61%, [109, 179]), while dogs (15%, [26/179]) and chickens/ducks (14%, [25/179]) were common (Table C9). We found the presence of chickens or ducks was associated with a greater number of pathogenic bacteria in latrine entrance soils (aPR = 1.8, [1.4, 2.4]), but was not associated with the number of pathogenic viruses, protozoa, or STHs (Table 16). In adjusted models, the presence of dogs was associated with a greater number of pathogenic viruses (aPR = 1.8, [1.2, 2.8]), but not with the number of pathogenic bacteria, protozoa or STHs (Table 16). Though the association between dogs and the number of pathogenic viruses was not significant after adjusting for multiple comparisons. We observed no associations between the presence of cats and the number of any pathogenic taxa.

6.4.9.7 Visible feces

During the baseline and 24-month MapSan compound surveys we observed human or animal feces at approximately one-third of compounds (29%, [52/179]). In the unadjusted estimate visible feces was associated with an increased number of pathogenic viruses (PR = 1.5, [1.0, 2.3]), but not in the adjusted estimate (aPR = 1.3, [0.84, 1.9]) and the unadjusted estimate was not significant after adjusting for multiple comparisons. Further, visible feces in the compounds was associated with a

greater number of STHs in latrine entrance soils (aPR = 1.3, [1.0, 1.8]), but not significantly after adjusting for multiple comparisons, and was not associated with the number of pathogenic bacteria or protozoa (Table 16).

6.4.9.8 Full pits

During the 24-month phase we observed if an on-site sanitation system was full. If the sludge was not visible, household members were asked if their pit was full or not. Nearly all compounds did not have a full pit (81%, [71/88]) while only two compounds – both of which were control pit latrines without slabs – had full pits (2.3%, [2/88]). Whether a pit was full was indeterminable at some compounds (17%, 15/88), and as such we excluded indeterminable responses from the regression models. Despite the small number of full pits, the presence of a full pit was associated with a greater number of pathogenic bacteria (aPR = 3.8, [1.8, 8.4]), protozoa (aPR = 3.2 [1.3, 7.6]), and STHs (aPR = 1.7, [1.1, 2.5]) in latrine entrance soils, but not with a greater number of viruses (aPR = 1.6 [0.64, 3.8]). Though the associations with the number of viruses and STHs were not significant after adjusting for multiple comparisons.

Table 16. Factors that were associated with the number of pathogens detected in latrine entrance soils

	Number of	pathogenic bacteria		pathogenic viruses		pathogenic protozoa		STHs	
Independent Variable	Reference	Unadjusted PR	Adjusted PR	Unadjusted PR	Adjusted PR	Unadjusted PR	Adjusted PR	Unadjusted PR	Adjusted PR
Sanitation: open defecation or neighbor's latrine	Pit latrine without a slab	**0.31 (0.16, 0.59)**	**0.29 (0.15, 0.57)**	0.81 (0.21, 3.2)	0.82 (0.30, 2.3)	1.1 (0.30, 3.7)	1.2 (0.40, 3.5)	0.89 (0.49, 1.6)	0.93 (0.54, 1.6)
Sanitation: non-intervention pour-flush toilet		0.73 (0.40, 1.3)	0.76 (0.45, 1.3)	0.21 (0.06, 0.74)	0.22 (0.06, 0.81)	0.88 (0.34, 2.3)	1.1 (0.41, 2.9)	0.77 (0.49, 1.2)	0.93 (0.60, 1.4)
Sanitation: intervention pour-flush toilet		**0.61 (0.43, 0.88)**	**0.57 (0.39, 0.82)**	0.71 (0.44, 1.2)	0.62 (0.37, 1.0)	0.81 (0.53, 1.2)	0.78 (0.49, 1.3)	**0.59 (0.40, 0.86)**	**0.56 (0.38, 0.83)**
Sanitation: pit latrine with a slab		0.82 (0.60, 1.1)	0.79 (0.58, 1.1)	0.89 (0.59, 1.3)	0.91 (0.59, 1.4)	1.3 (0.87, 1.9)	1.3 (0.84, 1.8)	0.80 (0.60, 1.1)	0.79 (0.59, 1.1)
Compound population	10-person increase	1.2 (1.0, 1.4)	1.2 (1.0, 1.4)	**1.4 (1.1, 1.7)**	1.3 (1.0, 1.6)	1.3 (1.1, 1.6)	1.3 (1.0, 1.6)	1.2 (1.0, 1.4)	1.1 (0.94, 1.3)
Visibly wet soil	Visibly dry soil	1.1 (0.83, 1.6)	1.2 (0.85, 1.6)	1.2 (0.77, 1.9)	1.2 (0.76, 1.8)	1.2 (0.73, 2.0)	1.3 (0.73, 2.3)	1.2 (0.88, 1.8)	1.3 (0.93, 1.7)
Wealth index	1-quartile increase	1.0 (0.91, 1.2)	1.0 (0.86, 1.2)	0.87 (0.74, 1.0)	0.89 (0.75, 1.1)	0.92 (0.79, 1.1)	0.88 (0.74, 1.1)	0.99 (0.88, 1.1)	0.94 (0.83, 1.1)
Temperature	10^{o} F increase	0.85 (0.60, 1.2)	0.96 (0.68, 1.4)	0.81 (0.55, 1.2)	0.86 (0.58, 1.3)	0.72 (0.46, 1.1)	0.70 (0.43, 1.1)	0.78 (0.57, 1.1)	0.83 (0.62, 1.1)
Chicken(s) present	No chicken(s)	**1.8 (1.4, 2.3)**	**1.8 (1.4, 2.4)**	1.1 (0.68, 1.8)	1.0 (0.67, 1.5)	1.2 (0.70, 2.0)	1.2 (0.70, 2.1)	1.2 (0.89, 1.7)	1.3 (0.97, 1.7)
Dog(s) present	No dog(s)	1.3 (0.96, 1.7)	1.2 (0.90, 1.6)	1.7 (1.1, 2.7)	1.8 (1.2, 2.8)	1.1 (0.67, 1.8)	1.0 (0.62, 1.7)	1.4 (0.98, 1.9)	1.3 (0.95, 1.7)
Cat(s) present	No cat(s)	1.1 (0.82, 1.5)	1.1 (0.82, 1.5)	1.2 (0.79, 1.7)	1.1 (0.76, 1.6)	1.1 (0.78, 1.6)	1.1 (0.75, 1.6)	1.2 (0.93, 1.6)	1.2 (0.91, 1.6)
Visible feces	No visible feces	1.2 (0.90, 1.6)	1.1 (0.80, 1.4)	1.5 (1.0, 2.3)	1.3 (0.84, 1.9)	1.3 (0.94, 1.9)	1.11 (0.76, 1.6)	**1.5 (1.2, 1.9)**	1.3 (1.0, 1.8)
Pit full	Pit not full	**4.1 (2.9, 5.9)**	**3.8 (1.8, 8.3)**	**2.9 (1.6, 5.1)**	1.6 (0.64, 3.8)	**2.7 (2.0, 3.8)**	3.2 (1.3, 7.6)	**2.7 (2.2, 3.3)**	1.7 (1.1, 2.5)

Note: Bold indicates $p \leq 0.05$ after false discovery rate correction for multiple comparisons across taxa (i.e. the correction was applied across each column). PR: prevalence ratio. STH: soil-transmitted helminth. F: Fahrenheit.

6.5 DISCUSSION

We found evidence that this on-site shared urban sanitation intervention was protective against the prevalence and number of bacteria detected in latrine entrance soils, but no evidence that it was protective against the prevalence or number of pathogenic viruses, protozoa, or STHs. Though our small sample size yielded some confidence intervals that crossed the null, DID estimates for the six most common individual bacteria were consistently protective (point estimate range: 0.10-0.63), which suggests the intervention may have reduced these pathogenic bacteria in latrine entrance soils. While the MapSan trial did not test for EAEC or EPEC in children's stools, *Shigella* spp. was evaluated and was the most common bacterial pathogen at baseline.[78] As the most prevalent bacterial pathogen, the trial had greater power to observe an effect on *Shigella* spp. than other bacterial pathogens. In fact, for children born into study compounds before the 24-month visit, the intervention reduced the prevalence of *Shigella* spp. in children's stools by 51%.[43] The 54% reduction in *Shigella* spp. prevalence we observed in soils agrees with the enteric infection data for children evaluated by the MapSan trial. The predictors of the number of pathogens soils that we observed further supports the evidence from our difference-in-difference analysis. Compared to pit latrines without slabs, we found that intervention pour-flush toilets were associated with a reduced number of pathogenic bacteria and STHs in latrine entrance soils. The observed DID estimates, combined with the associations from our predictor analysis, might suggest that the intervention reduced the spread of pathogenic bacteria to latrine entrance soils, and subsequently contributed to a reduction in children's intra-compound exposures to *Shigella* spp. and perhaps other bacterial pathogens, but our

small sample size and imprecise estimates suggest results should be interpreted with caution.

Compared to other large-scale, rigorous trials of sanitation in rural Bangladesh (pour flush to double-pit latrine)[38], rural Kenya (single unlined pit latrine with plastic slab and hole-lid)[39], and rural Zimbabwe (ventilated improved pit latrine)[40], we evaluated a more substantial on-site sanitation intervention (pour-flush to septic tank with drain field)[78,92] in an urban setting. Despite reductions in other environmental compartments (stored drinking water and hand contamination), WASH Benefits (WASH-B) Bangladesh did not find significant reductions in culturable *E. coli*[217], enteric pathogens[54] or microbial source tracking markers (HumM2, BacCow)[54] in household entrance soils. Likewise, WASH-B Kenya found the intervention reduced culturable *E. coli* in stored drinking water, but not along other transmission pathways.[53] Similar to the WASH-B Kenya intervention which upgraded latrines without slabs to latrines with plastic slabs, we observed no association between pit latrines with slabs and the number of pathogenic bacteria, viruses, protozoa, and STHs in latrine entrance soils compared to latrines without slabs. The (SHINE) trial in rural Zimbabwe did not evaluate the intervention's impact on environmental fecal contamination. This study is the first controlled evaluation of an urban on-site sanitation intervention to show a decrease in the prevalence of bacterial pathogens in latrine entrance soils.

The intervention may have reduced the spread of fecal contamination to the environment compared to controls because the intervention may have better sequestered fecal material, been easier to clean, or increased the likelihood of hygienic pit emptying. Pit latrines in low-income Maputo are often covered when full and rebuilt, or the fecal sludge is emptied

and buried nearby.[207] The intervention sanitation systems represented an upgrade to a more permanent sanitation infrastructure. The high-quality construction, which included cinder block walls, a cement floor, cinder block lined septic tank, cinder block lined drain field, tin roof, and a water seal squat pan may have better sequestered fecal contamination than control systems. Intervention systems also contained a drain for bathing, which may have prevented fecally contaminated graywater from spreading into nearby soils, and the cement floors were likely easier to clean than control systems with dirt floors. In addition, the intervention included programming to encourage hygienic pit emptying and provided equipment and training to local organizations to offer hygienic emptying services.[92] During the 24-month phase, intervention compounds emptied their sanitation systems less frequently and were more likely to have hygienically emptied their on-site systems than control compounds.[207] Less frequent emptying would have created fewer opportunities for the spread of fecal contamination and hygienic emptying may have reduced the quantity of fecal sludge that spread into soils during emptying. Indeed, the observed association between full pits and a greater number of bacteria, protozoa and STHs in latrine entrance soils further indicates the importance of hygienic and timely pit emptying to reduce the spread of fecal contamination.

On one hand, we less frequently detected some individual pathogens, such as *Shigella* spp. and EPEC, in intervention soils compared to controls during the 24-month phase. On the other, two years after the intervention we still detected one or more fecal pathogens in 86% of intervention latrine entrance soils. Animal[5] and children's feces[218], lack of community coverage, and a high population density[6] were not addressed by the intervention and should be considered as other factors that affect the spread of environmental fecal contamination

to soils. While we adjusted for animals in our DID estimates, many animals are not penned in low-income Maputo and may defecate outside of their respective compounds. The movement of some animals, such as dogs which were associated with an increased number of viruses in soils, may have reduced our power to observe an effect on some pathogens. Some young children practiced open defecation and the disposal of feces into a latrine was rare (6.4%) for children 1-23 months old at baseline.[78] The presence of visible feces in the compound, from humans or animals, was associated with an increased number of STHs. In addition, the intervention was not intended to achieve any threshold of sanitation coverage in study neighborhoods where the population density exceeds 15,000 people per square kilometer.[6] Increasing compound population was associated with a greater number of pathogenic bacteria, viruses, and protozoa. A higher threshold of sanitation coverage may be necessary to further reduce environmental fecal contamination in this urban setting, though complete basic sanitation coverage in rural Bangladesh was not associated with a significant decrease in environmental fecal contamination.[219]

We may have observed an effect on the prevalence and number of pathogenic bacteria detected, but not other pathogenic taxa for a variety of reasons. First, we tested for ten pathogenic bacteria, but only five viruses, three protozoa, and two helminths. Further we detected bacteria more often than other pathogenic taxa. This difference resulted in a greater power to detect an impact on bacteria than other taxa and may explain why we only observed an impact on pathogenic bacteria. In addition, viruses are often spread from person-to-person and WASH interventions may not interrupt their transmission, which may explain the observed null effect.[220,221] *Giardia duodenalis* – the most common protozoa we detected in soil – was the most common enteric infection among children 12-

48 months old during the MapSan trial baseline[78] and is a zoonotic pathogen capable of infecting dogs and cats.[222] The ubiquitous presence of *Giardia duodenalis* in this setting may have prevented us from observing an effect of the intervention on the prevalence or number of pathogenic protozoa in latrine entrance soils. *Ascaris* and *Trichuris* may remain infectious in soils for months or years depending on environmental conditions.[223,224] Considering our two-year follow-up period, in similar high population density settings where the burden of STH infection is high[78], additional interventions such as mass deworming campaigns may be necessary to reduce STH infections and the resulting potential spread of STH ova into soils. However, there was evidence the intervention might have reduced the prevalence of any STH and *Trichuris* infection among children born into intervention sites after implementation.[43]

The similar reduction in *Shigella* spp. prevalence in soils compared to children's stools from the MapSan birth cohort may be informative about children's exposures. During the MapSan 24-month phase, children born into study compounds were 1-24 months old, while children enrolled at baseline were 25-73 months old.[43] Considering the observed reduction in the prevalence of *Shigella* spp. in soils in this study, the dominant *Shigella* spp. exposure pathways for children 1-24 months old may be inside the compound. However, older children are more mobile than younger children, and their exposures to *Shigella* spp. outside of study compounds may explain why the intervention did not reduce the prevalence of *Shigella* spp. for these older children.

Our study had a relatively small sample size and was not powered to detect small reductions in the prevalence or number of pathogens in latrine entrance soils. Nevertheless, in high burden settings, WASH interventions may need to achieve a dramatic reduction in

environmental fecal contamination to reduce exposure risks and yield improved health outcomes.[58] The sample size was sufficient to indicate the intervention did not radically change the level of environmental fecal contamination. We conducted multiple hypothesis tests, which increased the possibility for type I errors and therefore used a false discovery rate correction.[225,226] In addition, all variables in our analysis have a strong foundation in the literature as factors that may contribute to the detection of enteric pathogens in latrine entrance soils. We assessed gene targets via molecular assays – which may have some error associated with them – and not pathogen viability or infectivity; some of the genes we detected may have come from environmental DNA/RNA[227] or non-infectious pathogens. However, these detections may represent a historical snapshot of the past performance of the sanitation infrastructure's ability to prevent the spread of fecal contamination into the environment, which may be desired in assessing the impact of a sanitation intervention. Although, in another study we found the prevalence and count of culturable *E. coli* was high in latrine entrance soils 24-months post-intervention[29], suggesting some of the pathogens we detected may have been viable.

There is substantial evidence that city-wide upgrades to sanitation infrastructure improve health outcomes.[11,228,229] However, the high capital and maintenance costs of such improvements suggests they are currently impractical for many LMICs. Until sewerage becomes feasible, on-site sanitation systems remain necessary to achieve safely managed sanitation. The results of this study – and other rigorous evaluations of the environmental impact of on-site sanitation interventions – suggest that fecal contamination spreads into the environment through multiple complex pathways that may vary between settings.[57] In urban Maputo – and similar settings with poor WASH infrastructure, ubiquitous

environmental fecal contamination, and a high burden of enteric infection – other interventions targeting hygienic fecal sludge management, drainage, housing, and solid waste management may need to accompany improvements to on-site sanitation infrastructure to reduce the spread of fecal contamination into the environment through site-specific pathways.

CHAPTER 7. A QUANTITATIVE MICROBIAL RISK ASSESSMENT OF PEDIATRIC INFECTIONS ATTRIBUTABLE TO INGESTION OF FECALLY CONTAMINATED DOMESTIC SOILS IN LOW-INCOME URBAN MAPUTO, MOZAMBIQUE

7.1 ABSTRACT

Rigorous studies of water, sanitation, and hygiene interventions in low- and middle-income countries (LMIC) suggest children are exposed to enteric pathogens via multiple complex pathways, which may include soil ingestion. At 30 compounds (household clusters) in low-income urban Maputo, Mozambique, we cultured *E. coli* and quantified gene targets from soils (*E. coli*: *ybbW*, *Shigella* spp.: *ipaH*, *Giardia duodenalis*: *beta-giardin*) using droplet digital PCR at three compound locations (latrine entrance, solid waste area, dishwashing area). We found 88% of samples were positive for culturable *E. coli* (mean = $\log_{10}$ 3.2 CFUs per gram of dry soil), 100% for molecular *E. coli* (mean = $\log_{10}$ 5.9 gene copies per gram of dry soil), 44% for *ipaH* (mean = $\log_{10}$ 2.5) and 41% for *beta-giardin* (mean = $\log_{10}$ 2.1). Performing stochastic quantitative microbial risk assessment using soil ingestion parameters from a LMIC setting for children 12-23 months old, we estimated the median annual infection risk by *Giardia duodenalis* from was 6,700-fold and by *Shigella* spp. was 3,700-fold greater than the EPA's standard for drinking water. Our results indicate compounds in Maputo – and similar settings – require contact and source control strategies to reduce the ingestion of contaminated soil and achieve levels of acceptable risk.

7.2 INTRODUCTION

In low- and middle-income countries (LMIC), children may be repeatedly exposed to and infected by enteric pathogens during the first years of life.[76] Exposures can lead to enteric infections, with or without diarrheal disease[24], and a range of hypothesized effects including poor growth[25], adverse cognitive development[26], negative effects on the immune system[27] and reduced efficacy of oral vaccines[28]. Rigorous studies of water, sanitation, and hygiene (WASH) interventions in LMICs have found mixed impacts on children's health, and multiple interrelated environmental pathways may commonly transmit enteric pathogens from feces to new hosts.[38–40,42,44,198] Across a diverse range of rural and urban settings in LMICs, ingestion of fecally contaminated soils is increasingly recognized as a route of exposure.[29,52,203,230,54,94,96,97,100,103,199,200] Enteric pathogens in soils may be directly ingested[102] or spread to hands, food, fomites, or household stored water before subsequent ingestion.[97] Some children may practice geophagy[200,231] – a form of pica involving soil ingestion - which has been associated with environmental enteropathy[232], stunting[232], and grow-faltering[233]. These exposures suggest that characterizing infection risks from soil ingestion may be useful to inform intervention strategies and reduce risks.

In several LMICs where safely managed sanitation is lacking, evidence suggests that fecal contamination is ubiquitous in soil.[204] Both fecal indicator bacteria (FIB) and gene targets from enteric pathogens have been detected – often at high number and prevalence – in soils from the domestic and public environments.[29,54,94,100,203,205] The presence of viable FIB and enteric pathogen gene targets suggests that infection risks from soil ingestion may be high.

Quantitative microbial risk assessment (QMRA) is a systematic, mechanistic, evidence-based framework for translating observations of potential microbial hazards into health risks.[104] Combining QMRA with stochastic methods propagates the variability and

uncertainty from model parameters to demonstrate the potential range of expected risks. Whereas epidemiologic studies often require large sample sizes to detect differences in low frequency outcomes – and subsequently are expensive – QMRA offers an alternative approach to estimate infection risks. As such, QMRA has often been used to characterize the risk from activities with a low independent probability of infection, such as consumption of contaminated drinking water[106,234,235] or ingestion of surface water during recreational activities[108,109,235].

Given the increasing attention to fecally contaminated soils in the literature, some QMRA models have investigated the potential infection risks posed by soils.[110,111] However previous, soil-focused QMRA models applied to LMICs were often not stochastic[110,111], assumed 100% pathogen viability[110], assumed a large amount of soil ingested per dose[111] (e.g. five grams of soil), or did not include a sensitivity analysis[110,111]. In addition, we know of no paper that used enteric infection prevalence in a LMIC to assess if the output of a QMRA model for soil ingestion was reasonable.

The data for the current QMRA were collected as part of the Maputo Sanitation (MapSan) trial, a non-randomized controlled trial that assessed the impact of a shared on-site sanitation intervention on children's health in low-income urban Maputo, Mozambique. The primary outcome in the MapSan trial was the prevalence of bacterial or protozoan infection as indicated by children's stool, measured by a multiplex reverse transcriptase PCR assay[78]. The aims of our assessment were to: (1) use QMRA to assess the annual risk of infection by *Shigella* spp. and *Giardia duodenalis* from ingestion of fecally contaminated soils in the domestic environment in the MapSan trial cohort, (2) use sensitivity analysis to investigate the correlations between input parameters and estimated

risks, and (3) compare the model output with the age-stratified prevalence of *Shigella* spp. and *Giardia duodenalis* among children enrolled in the MapSan trial. We focused on *Shigella* spp. and *Giardia duodenalis* as these were the most prevalent bacterial and protozoan enteric pathogens identified in the MapSan trial during the 24-month phase[43], present in approximately 55% (95% CI: 53% - 59%) and 63% (60% - 66%) of stools from all children enrolled in this cohort.

7.3 METHODS

7.3.1 Sample Selection

From 80 MapSan compounds assessed from May-June 2018 as part of a previous study,[29] we randomly selected 15 MapSan control and 15 intervention compounds for inclusion in this study. As described previously[29], we collected soils from standardized compound locations and assessed culturable *E. coli* counts using Compact Dry Plates (Compact Dry™ EC, VWR, Vienna, Austria). We shipped aliquots of these samples from the Mozambican National Institute of Health in Maputo, Mozambique to Georgia Institute of Technology in Atlanta, GA on dry ice (-80°C) with temperature monitoring. We selected three compound soil locations for molecular analysis for a total of 90 soil samples. Sample locations included the point 0.25 meters directly in front of: (1) the latrine entrance; (2) the solid waste storage area (solid waste was typically stored in a rice sack or was uncontained on the ground and was always outside in the shared common space); (3) the outside area where compound members most frequently washed their dishes (e.g. we typically sampled where people would stand or squat while washing their dishes). When cement flooring was present, we sampled the nearest point not covered by cement. We selected these locations

for standardization across compounds based on three plausible scenarios for the spread of fecal contamination into domestic soils. Latrine entrance soils may receive an input of fecal material from latrines that inadequately sequester fecal wastes, while soils at solid waste storage areas may be contaminated from the improper disposal of children's feces or other fecally contaminated solid wastes, including animal feces. However, soils at dishwashing areas have no similar point source of fecal contamination, but instead may receive fecal wastes from various sources or mechanisms that contribute to fecal contamination in the domestic environment (e.g. yard cleaning, walking, and wind). Altogether, we assumed that the pathogen distribution from these three locations would be representative of the domestic soils children ingest in this setting.

7.3.2 Sample Processing

We incubated a 500 mg aliquot of each soil sample at 105ºC for 1 hour to determine moisture content[212], discarded the dry soil, then extracted DNA from a separate 1-gram portion of each sample (dry weight). Following the manufacturer's protocol, we extracted DNA using the RNeasy PowerSoil DNA Elution Kit, and RNA with the RNeasy PowerSoil Total RNA Kit (Qiagen, Hilden, Germany). We spiked samples with MS2 as an extraction control and included one negative extraction control on each day of extractions (typically 15 samples per day).

7.3.3 Droplet digital PCR

We first tested for the presence of the extraction control MS2[236] using reverse transcription PCR on an ABI 7500 (Applied Biosystems, Foster City, CA), then quantified gene copies of *ybbW* (molecular *E. coli*)[237], *ipaH* (*Shigella spp.*)[238], and *beta-giardin* (*Giardia*

duodenalis assemblage B)[239] using droplet digital PCR with a QX200™ droplet reader (Bio-Rad Laboratories, Hercules, CA, USA) (Table D1). Following the probit method proposed by Stokdyk *et al*. 2016[240] we assayed a dilution series of g-blocks (Integrated DNA Technologies, Coralville, IA) for our *ipaH* and *beta-giardin* assays in triplicate to determine the 95% limit-of-detection (LOD) (Figure D1). We performed manual thresholding between positive and negative clusters taking into account the observed clusters in positive controls and extraction blanks to classify positive droplets.

7.3.4 Exposure Assessment

To model the distribution of *ipaH* and *beta-giardin* in soils, we used an imputation method using maximum-likelihood estimation to estimate distribution parameters.[241] Briefly, from our complete dataset of detects and non-detects, we imputed values for each non-detection observed by drawing from a uniform distribution from zero to the 95% LOD. We repeated this process 100 times to create 100 unique datasets. Then we used the *fitdistrplus*[242] package in R (R version 4.0.0, R Foundation for Statistical Computing, Vienna, Austria) to fit a log-normal distribution to the mean and standard deviation (SD) parameters from the 100 imputed datasets. As such, the final models were log-normal distributions for the density of *ipaH* and *beta-giardin* genes in domestic soils where the mean and standard deviation were themselves log-normal distributions.

Without site specific soil ingestion data, we developed QMRA models based on two plausible soil ingestion scenarios. First, we used parameters from the US EPA Exposure Factors Handbook[208], which are derived from studies of trace elements in feces and represent a low ingestion scenario from children living in Western-style housing. Secondly,

in order to represent a high ingestion scenario, we used parameters from Kwong *et al*. 2019, which directly observed children in low-income rural Bangladesh (Table 17). Recognizing that children's interaction with their environment varies with age, we disaggregate our risk estimates by age based on the available ingestion estimates from our two sources (Table 17). In addition, evidence suggests that some children practice geophagy[200,231]. Accordingly we include soil ingestion estimates for such children from the US EPA Exposure Factors Handbook.[208]

7.3.5 Dose harmonization and infectious unit

To estimate the proportion of viable *Shigella* spp. colony-forming units (CFU) and *Giardia duodenalis* cysts, we divided each soil sample's count of *E. coli* CFUs by its matched density of *ybbW* gene copies (Table 17). Then, we used maximum-likelihood estimation (MLE) (*fitdistrplus* package in R)[242] to fit a log-normal distribution to these ratios to use as an input for the proportion of viability in our QMRA model (Table 17).

In addition, *Shigella* spp. CFUs and *Giardia duodenalis* cysts contain multiple gene copies of our target sequences. To account for this in our models, we included a uniform distribution (5-14 gene copies *ipaH* / CFU) for *Shigella spp.*[238] and a static input (16 gene copies *beta-giardin* / cyst) for *Giardia duodenalis*[243] (Table 17).

7.3.6 Dose response

We estimated the probability of infection with *Shigella* spp. using the approximate beta-Poisson model with the log of parameters alpha and the median infectious dose using normal distributions (Table 17, Appendix D.1 Equations used in QMRA model).[244,245]

Likewise, we estimated the probability of infection with *Giardia duodenalis* using an exponential model with parameter k log-normally distributed (Table 17, Appendix D.1 Equations used in QMRA model).[246]

Table 17. Input parameters for QMRA model

Model variable	Stochastic parameters used	Reference
Exposure Assessment		
non-detect values of *ipaH* and *beta-giardin* (gene copies per gram soil)	U(0, 95% LOD)	Canales *et al.* 2018[241]
gene copies *beta-giardin* per gram dry soil	LN distribution with the following LN parameter distributions: mean = LN (1.5, 0.019) sd = LN (0.45, 0.076)	MLE, this study
gene copies *ipaH* gene per gram dry soil	LN distribution with the following LN parameter distributions: mean = LN (1.7, 0.016) sd = LN (0.41, 0.078)	MLE, this study
Soil ingested (grams/day) (EPA 2011)	<6 months: LN(-4.2, 0.78) (mean = 20 mg/day, sd = 18 mg/day) 6-11 months: LN(-4.0, 0.95) (mean = 30 mg/day, sd = 36 mg/day) 12-23 months: LN(-3.4, 0.68) (mean = 40 mg/day, sd = 30 mg/day) 24-71 months: LN(-4.0, 0.95) (mean = 30 mg/day, sd = 36 mg/day) Geophagy (12-71 months): U(1, 50)	EPA Exposure Factors Handbook[208]

Soil ingested (grams/day) (Kwong *et al.* 2019)	3-5 months: LN(-1.8, 0.69) (geometric mean = 162 mg/day, geo sd = 2) 6-11 months: LN(-1.5, 0.69) (geometric mean = 224 mg/day, geo sd = 2) 12-23 months: LN(-1.5, 0.69) (geometric mean = 234 mg/day, geo sd = 2) 24-35 months: LN(-1.8, 0.69) (geometric mean = 168 mg/day, geo sd = 2) 36-47 months: LN(-1.7, 0.69) (geometric mean = 178 mg/day, geo sd = 2)	Kwong *et al.* 2019[102]

Table 18 continued

Dose harmonization and infectious unit		
Culturable *E. coli* in intra-compound soils (log_{10} CFU / gram of dry soil)	N (3.2, 1.1)	This study
ybbW in intra-compound soils (log_{10} gene copies / gram of dry soil)	N (5.9, 0.36)	This study
Ratio of viable *Shigella* spp. CFUs: Proportion of culturable *E. coli* to molecular ybbW GC	LN(-6.2, 2.4) LN distribution from ratios of CFUs *E. coli* to *ybbW* (truncated at 1)	MLE, this study
Ratio of viable *Giardia* cysts	LN(-6.2, 2.4) LN distribution from ratios of CFUs *E. coli* to *ybbW* (truncated at 1)	MLE, this study
beta-giardin gene copies per cyst	16	Bernander *et al*. 2001[243]
i*paH* gene copies per CFU	U(5,14)	Lin *et al*. 2010[238]
ybbW gene copies per *E. coli* genome	1	Walker *et al*. 2017[237]
Dose-Response		
Giardia duodenalis dose response parameter, k	LN (0.0208, 0.0064)	Rose *et al*. 1991[246]
Shigella spp. dose-response parameters, alpha, N_{50}	log α N(-0.5768, 0.0961) log N_{50} N(3.170, 0.1397)	Dupont et al. 1972[244] Crockett *et al*. 1996[245]

Note: CFU: Colony forming unit. SD: standard deviation. GC: gene copies. LN: lognormal distribution (mean, sd). N: normal distribution (mean, sd). U: uniform distribution (min, max). Reported parameters for distributions correspond to the inputs for the *rlnorm*, *rnorm*, and *runif* functions in R.

7.3.7 Risk characterization

To propagate the uncertainty and variability from the stochastic input distributions into risk estimates, we programmed the model as a Monte Carlo simulation in R version 4.0.0, where we randomly sampled from each stochastic distribution in independent trials, then calculated the daily risk of infection for each draw (Appendix D.1 Equations used in QMRA model). We executed the model by running 10,000 independent trials. To calculate an annual probability of infection we sub-sampled 365 daily probabilities from the 10,000

generated by the model without replacement, and calculated the annual probability of infection using equation two.[247] We bootstrapped the model by repeating this process 10,000 times, which we used to calculate summary statistics. To ensure reproducibility, we standardized all Monte Carlo simulations in R with an initial seed value of 31.

$$P_{inf,annual} = 1 - \prod_1^n (1 - P_{inf,daily,i}) \, , n = 365 \qquad \text{(Equation 2)}$$

7.3.8 Sensitivity analysis

To assess the sensitivity of the estimated daily risk of infection with stochastic input parameters for models of children 12-23 months old we calculated the Spearman's rank correlation coefficient.[248]

7.4 RESULTS

7.4.1 Overview of observed fecal contamination

We found evidence of widespread fecal contamination across all three intra-compound locations (Table 19, Figure 3). We detected the *beta-giardin* gene in 41% (37/90) of samples, the *ipaH* gene in 44% (40/90) of samples, the *ybbW* gene in 100% of samples (90/90), and culturable *E. coli* in 88% (79/90) samples. Per gram of dry soil, observed densities of the *beta-giardin* gene (mean = $\log_{10}$ 2.1, sd = 0.61) and the *ipaH* gene (mean = $\log_{10}$ 2.5, sd = 0.52) were relatively homogenous across intra-compound locations and were substantially lower than the *ybbW* gene (mean = $\log_{10}$ 5.9, sd = 0.37). The median ratio of CFUs *E. coli* to gene copies of *beta-giardin* and *ipaH* was similar at all three intra-compound locations, but the overall range of ratios varied by about 5-$\log_{10}$ for both gene targets (Figure 4).

Table 19: Summary of molecular and culture-based assays. For reporting purposes all non-detects (ND) were set to half the 95% LOD for molecular assays and to half the LOD for the culture-based assay

Log_{10} transformed density of *beta-giardin* gene copies per gram of dry soil				
Location	Prevalence	Mean (sd)	Median	Range
Latrine entrance	47% (14/30)	2.1 (0.54)	ND	ND, 3.3
Solid waste	30% (9/30)	2.0 (0.51)	ND	ND, 3.4
Dishwashing	47% (14/30)	2.3 (0.73)	ND	ND, 3.8
Total	41% (37/90)	2.1 (0.61)	ND	ND, 3.8
Log_{10} transformed density of *ipaH* gene copies per gram of dry soil				
Location	Prevalence	Mean (sd)	Median	Range
Latrine entrance	57% (17/30)	2.6 (0.69)	2.2	ND, 4.9
Solid waste	30% (9/30)	2.4 (0.32)	ND	ND, 3.4
Dishwashing	47% (14/30)	2.5 (0.47)	ND	ND, 3.8
Total	44% (40/90)	2.5 (0.52)	ND	ND, 4.9
Log_{10} transformed density of *ybbW* gene copies per gram of dry soil				
Location	Prevalence	Mean (sd)	Median	Range
Latrine entrance	100% (30/30)	5.8 (0.33)	5.8	5.4, 6.8
Solid waste	100% (30/30)	5.7 (0.35)	5.7	4.9, 6.3
Dishwashing	100% (30/30)	6.1 (0.27)	6.1	5.5, 6.8
Total	100% (90/90)	5.9 (0.37)	5.9	4.9, 6.8
Log_{10} transformed count of CFU *E. coli* per gram of dry soil				
Location	Prevalence	Mean (sd)	Median	Range
Latrine entrance	87% (26/30)	3.0 (1.1)	3.2	ND, 5.3
Solid waste	87% (26/30)	3.2 (1.1)	3.0	ND, 5.2
Dishwashing	90% (27/30)	3.3 (1.1)	3.2	ND, 5.3
Total	88% (79/90)	3.2 (1.1)	3.2	ND, 5.3

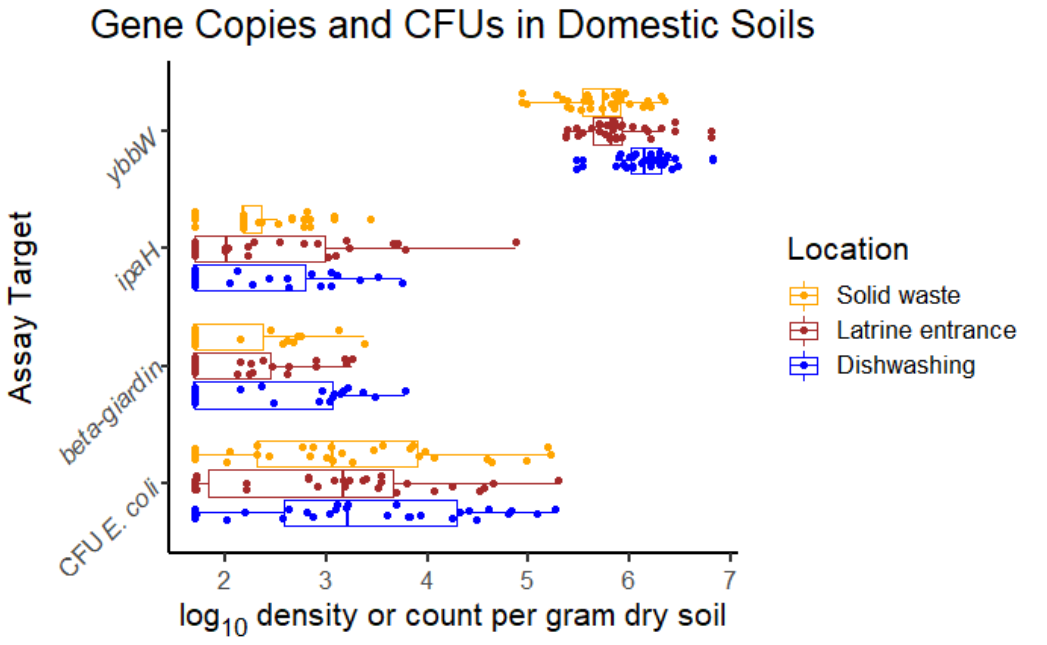

Figure 3. Results from molecular and culture-based assays

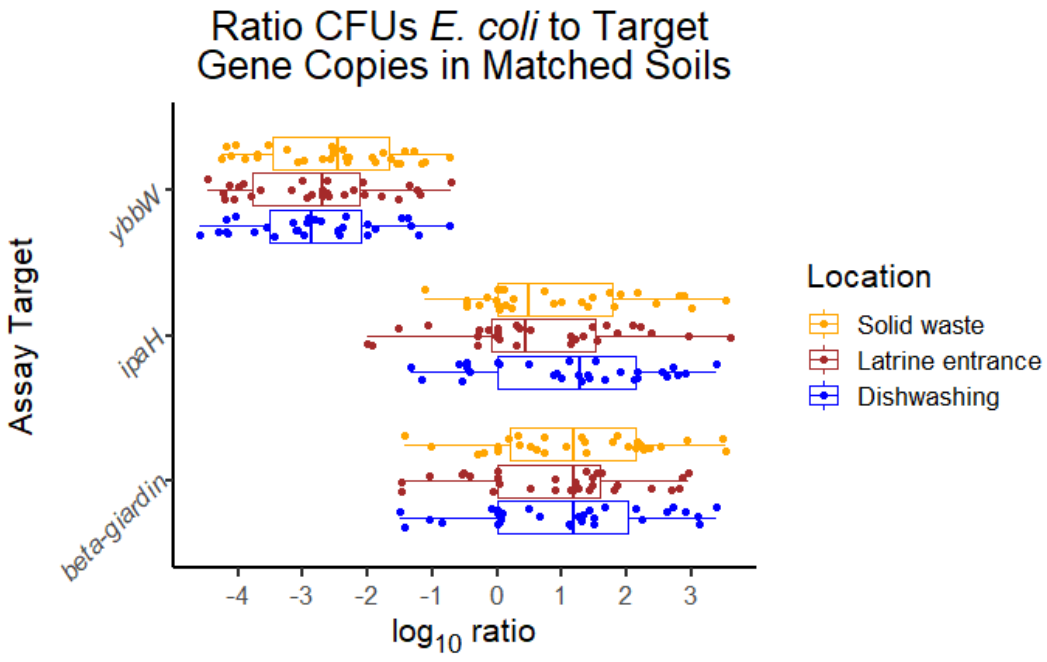

Figure 4. Ratios of CFUs E. coli to gene copies. Ratios calculated by dividing the count of CFUs *E. coli* by the gene copies of *ybbW*, *ipaH*, or *beta-giardin* in the matching soil sample

7.4.2 QMRA model output

7.4.2.1 Daily soil ingestion

Using parameters for the low ingestion scenario from US EPA 2011, we estimated children 12-23 months old ingest less soil per day (mean = 40 mg, standard deviation = 30 mg, median = 32 mg, range = 2-588 mg) than using soil ingestion parameters from Kwong *et al*. 2019 (mean = 302 mg, standard deviation = 240 mg, median = 235 mg, range = 19-4,500 mg).

7.4.2.2 Daily risk

For both pathogens under both ingestion scenarios the daily risk of infection was relatively low but was about an order of magnitude lower for the low ingestion rate scenario (Table D2, Figure D2). For example, using soil ingestion estimates from US EPA 2011, we estimated the median daily risk of infection for a child 12-23 months old by *Giardia duodenalis* was 1 in 110,000 and by *Shigella* spp. was 1 in 250,000. Using soil ingestion estimates from Kwong *et al*. 2019 we estimated the median daily risk of infection for a child 12-23 months old by *Giardia duodenalis* was 1 in 15,000 and by *Shigella* spp. was 1 in 32,000.

7.4.2.3 Annual risk

Regardless of age or soil ingestion scenario, we estimated the 10^{th} percentile of the annual risk of infection for both *Giardia duodenalis* and *Shigella* spp. was greater than the US Environmental Protection Agency's (EPA) normative standard for drinking water (≤1 in 10,000 infection risk per year). For children 12-23 months old – using ingestion estimates from Kwong *et al*. 2019 – the median annual risk of infection by *Giardia duodenalis* was 6,700-fold and by *Shigella* spp. was 3,700-fold greater than the EPA's standard for drinking water (

Table 20). As expected – because the mean amount of soil ingested was greater – the estimated annual risks were substantially higher using soil ingestion estimates from Kwong *et al*. 2019 compared to the US EPA Exposure Factors Handbook (Figure 3, Figure 4). Likewise, children practicing geophagy had the highest estimated annual risks (

Table 20).

Table 20. Estimated annual infection risks

	Model output using soil ingestion estimates from US EPA Exposure Factors Handbook				
	Estimated annual risk of *Giardia duodenalis* infection				
	Percentile	**10th**	**50th**	**90th**	**MapSan prevalence at 24-month phase**
Age	<6 months	4.3%	7.0%	15%	13%
	6-11 months	6.1%	11%	28%	22%
	12-23 months	8.5%	14%	23%	59%
	24-71 months	6.0%	9.8%	19%	73%
	Geophagy (12-71 months)	>99%	>99%	>99%	70%
	Estimated annual risk of *Shigella* spp. infection				
Age	<6 months	2.0%	3.3%	6.7%	5.0%
	6-11 months	2.8%	4.6%	8.4%	21%
	12-23 months	3.9%	6.3%	11%	36%
	24-71 months	2.7%	4.7%	9.1%	68%
	Geophagy (12-71 months)	>99%	>99%	>99%	62%
	Model output using soil ingestion estimates from Kwong *et al*. 2019				
	Estimated annual risk of *Giardia duodenalis* infection				
	Percentile	**10th**	**50th**	**90th**	**MapSan prevalence at 24-month phase**
Age	3-5 months	36%	53%	81%	13%
	6-11 months	47%	65%	88%	22%
	12-23 months	59%	67%	94%	59%
	24-35 months	37%	54%	75%	72%
	36-47 months	40%	59%	80%	75%
	Estimated annual risk of *Shigella* spp. infection				
Age	3-5 months	18%	27%	42%	5.0%
	6-11 months	23%	33%	48%	21%
	12-23 months	26%	37%	53%	36%
	24-35 months	19%	28%	44%	56%
	36-47 months	20%	29%	43%	73%

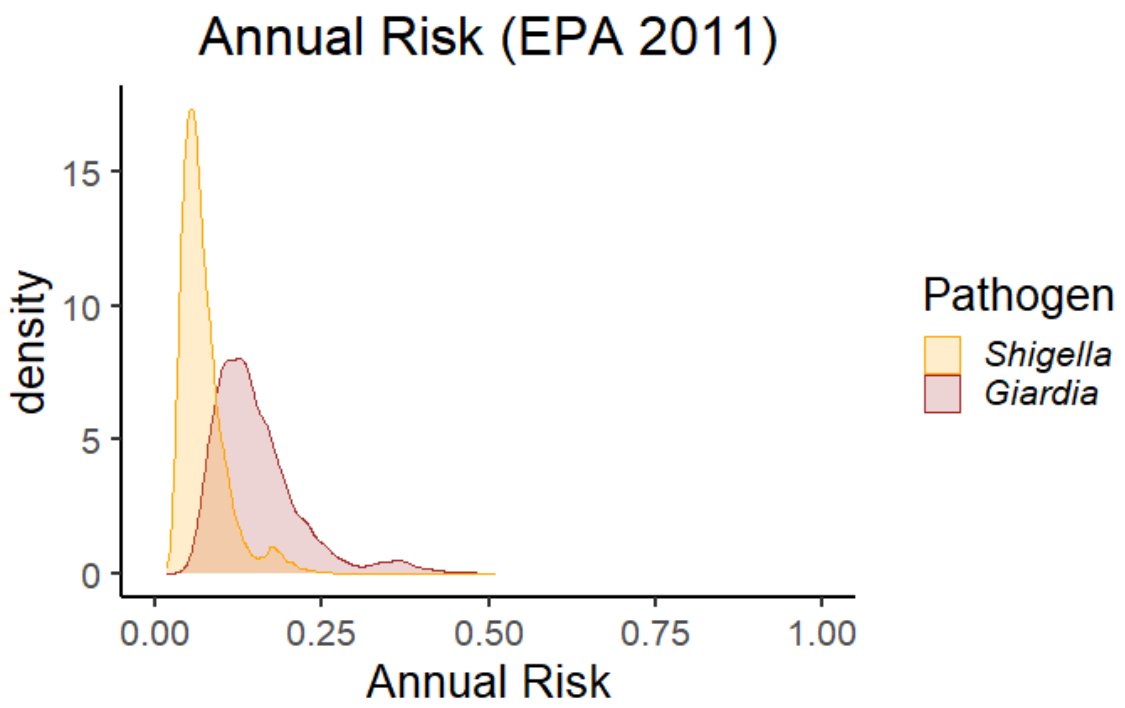

Figure 5. Kernel density plot of the estimated annual risk of infection using ingestion parameters from EPA 2011

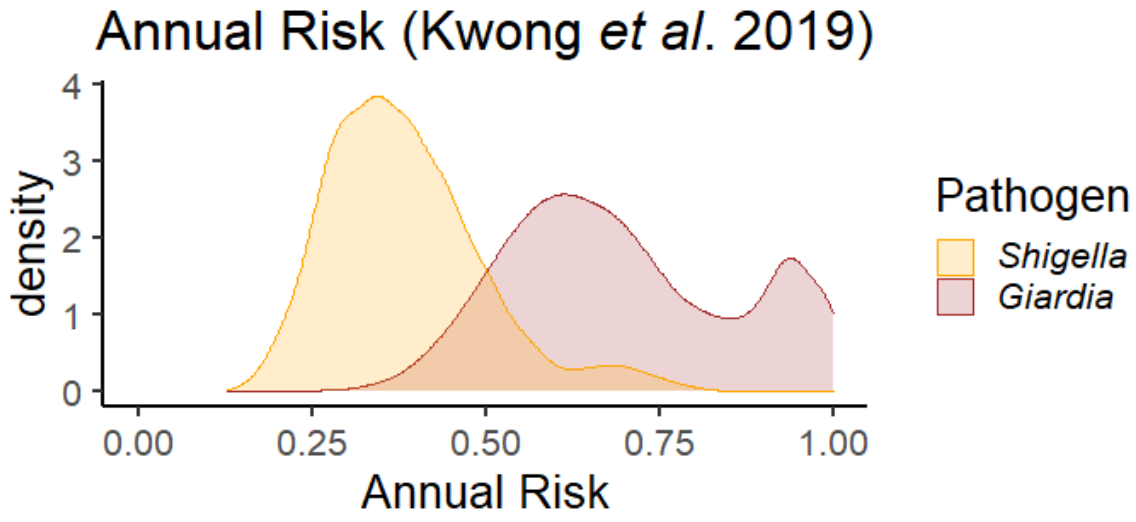

Figure 6. Kernel density plot of the estimated annual risk of infection using ingestion parameters from Kwong *et al.* 2019

7.4.3 Sensitivity analysis

We assessed the sensitivity of the estimated daily infection risks to stochastic input parameters for children 12-23 months old (Table 21). For both pathogens and both ingestion scenarios, the proportion of viable *Giardia duodenalis* cysts or *Shigella* spp. CFUs was strongly correlated with the daily risk of infection, and the density of *beta-giardin* or *ipaH* gene copies was moderately correlated with the daily risk of infection. For both pathogens, the dose response parameters were weakly correlated with the daily risk of infection. In the high-ingestion scenario (Kwong *et al*. 2019), there was no correlation between the amount of soil ingested and the daily risk of infection with either pathogen, while in the low ingestion scenario (EPA 2011) there was a weak correlation between the amount of soil ingestion and the daily risk of infection with either pathogen.

Table 21. The sensitivity of the daily estimated risk of infection with *Giardia duodenalis* or *Shigella* spp. for children 12-23 months old to input parameters as assessed by rank order correlation

Correlation with daily risk of infection by *Giardia duodenalis*		
	High ingestion (Kwong *et al*. 2019)	Low ingestion (US EPA 2011)
Input Variable	Spearman's Rank Correlation Coefficient	
Proportion of viable cysts viability (%)	0.77	0.78
beta-giardin density (gc/gram)	0.55	0.55
Dose response parameter, k	0.09	0.10
Soil ingestion (grams / day)	0.01	0.22
Correlation with daily risk of infection by *Shigella* spp.		
Input Variable	Spearman's Rank Correlation Coefficient	
Proportion of viable CFUs (%)	0.79	0.80
Ipah density (gc/gram)	0.50	0.50
Soil ingestion (grams / day)	0.01	0.22
Dose response parameter, N_{50}	-0.10	-0.11
Dose response parameter, alpha	-0.14	-0.14

7.5 DISCUSSION

We found evidence of widespread fecal contamination in domestic soils in low-income urban neighborhoods of Maputo, Mozambique. Regardless of the soil ingestion scenario used, the infection risks from children's ingestion of domestic soils contaminated by *Giardia duodenalis* and *Shigella* spp. were high compared to acceptable levels of risk for drinking water. Estimated annual infection risks were lowest using ingestion parameters from a high-income setting (US EPA 2011), higher using ingestion parameters from a low-income setting (Kwong *et al*. 2019), and highest for children practicing geophagy. In both ingestion scenarios, infection risk increased with age, peaked for children 12-23 months old, then decreased. We performed a sensitivity analysis and found for each model the proportion of viable cysts or CFUs was most strongly correlated with the daily risk of infection, the density of pathogen gene copies was moderately correlated, and the quantity of soil ingested had weak to no correlation. However, we estimated substantially lower infection risks using soil ingestion parameters from US EPA 2011 compared to Kwong *et al*. 2019. These findings suggest that interventions that reduce the ingestion of fecally contaminated soils – whether by source control (e.g. reduction of open defecation, improved latrines, or improved hygienic pit emptying)[207] or contact control (e.g. safe child play spaces or upgrading dirt floors to concrete)[249] – may be useful to reduce the risk of infection by *Giardia duodenalis* and *Shigella* spp. in this setting.

There are variety of factors that may contribute to the widespread fecal contamination detected in soil. Most neighborhoods in this setting have a population density greater 15,000 people per square kilometer and subsequently produce large amounts of human feces in a small geographic area.[6] Open defecation by young children and the unsafe

disposal of children's feces is common.[78] Furthermore, pit latrines and septic tanks are often emptied unhygienically using manual equipment and the fecal wastes buried on-site.[207] Animals are also commonly owned including cats, dogs, chickens, and ducks.[29,78] We tested for *Giardia duodenalis* assemblage B which can infect dogs and humans.[250,251] In addition, it is common for people to sweep the soil surface in the shared compound living space each morning, which may help spread pathogens across domestic soils in this setting.[252]

The dose of pathogens ingested is a product of the pathogen concentration and the quantity of soil ingested. This mathematical relationship offers two potential risk reduction strategies; source control may lower pathogen concentration and contact control may lower the quantity of soil ingested. As children are likely to ingest small amounts of soil each day, and we detected a high prevalence and density of pathogen associated gene copies in soil, it is logical that the proportion of viable cysts or CFUs ingested – and thus capable of causing an infection – were most strongly correlated with the daily risk of infection in both models. Importantly, both the proportion of viable cysts or CFUs and the density of pathogen gene copies in domestic soils may be reduced by source control. In addition, the median infectious dose of *Shigella* spp. (N_{50}=1,480 CFU)[244,245] is 42 times greater than *Giardia duodenalis* (N_{50}=35 cysts)[246], but the observed concentrations of both pathogens were generally equivalent to tens or hundreds of pathogens per gram soil. Considering *Giardia duodenalis* assemblage B is zoonotic[250,251] and has a low median infectious dose, a comprehensive intervention targeting both contact control and source control may be necessary to reduce infection risks in low-income Maputo. Though, where source control of animal feces is unworkable, contact control may be the preferred approach for zoonotic

pathogens such as *Giardia duodenalis*. On the other hand, *Shigella* spp. is human specific[253] and has a relatively high median infectious dose, suggesting that source control alone may reduce infection risks from soil ingestion in this setting.

While we observed a substantial difference in the estimated infection risks between the two soil ingestion rate scenarios, our sensitivity analysis revealed little to no correlation between the quantity of soil ingested and the daily infection risk within each ingestion scenario. As the impact of contact control on children's ingestion of soil in LMICs is not well characterized, it is possible some contact control interventions may not decrease infection risks. For example, if contact control – such utilization of safe child play spaces for a few hours a day – reduces the maximum possible amount of soil children ingest per day (e.g. from 3 grams to 1 gram), but does not reduce the median amount of soil ingested, then our model suggests it is unlikely the intervention will reduce infection risks. However, if soil ingestion in this setting is similar to quantities defined by Kwong *et al*. 2019, then a dramatic reduction in soil ingestion to the parameters defined in EPA 2011 would likely reduce infection risks from *Giardia duodenalis* and *Shigella* spp.

We observed a wide range for the ratio of the count of CFUs *E. coli* to the density of *ipaH* and *beta-giardin* gene copies, providing additional evidence that culturable *E. coli* is an imperfect indicator of *Shigella* spp. and *Giardia duodenalis* in soils.[101] This suggests previous QMRA approaches that relied on *E. coli* to pathogen ratios to quantify risks from drinking water may not be applicable to soil ingestion[254,255], and the use of such ratios would likely introduce substantial bias.

Historically, ingestion of soil has received less attention than other fecal-oral pathways such as water and food (Figure S2).[256] Comparing our estimates of infection risk with the observed prevalence of *Giardia duodenalis* and *Shigella* spp. infections suggests that soil ingestion could comprise a substantial proportion of pathogen transmission in this setting, and therefore soils may be an understudied and underappreciated pathway in similar environments.

Increasing prevalence of bacterial and protozoan infections with age was demonstrated among children in the MapSan cohort[78] and in a large study conducted in eight LMICs[257]. We estimated the median annual risk of *Giardia duodenalis* infection was 53% for children 3-5 months old and was 67% for children 12-23 months old, an increase of 14-percentage points (Kwong *et al*. 2019 ingestion parameters). Between the same two age ranges the prevalence of *Giardia duodenalis* in stools increased from 13% to 59%, a 46-percentage point increase. Although the QMRA output is annual risk and infection prevalence was cross-sectional – preventing direct comparison – we offer several general explanations for why the observed infection prevalence increased substantially more than the estimated infection risk. First, it is possible that children 3-5 months old are more likely to clear infections than children 12-23 months. The guts of older children may have been subject to repeated enteric infections and may be more likely to have persistent infections.[50,258,259] Children who quickly clear infections would be less likely to test positive in a cross-sectional survey than children with persistent infections. Both *Giardia duodenalis* and *Shigella* spp. have demonstrated the capacity for persistent infections in some individuals.[260,261] Alternatively, ingestion of domestic soils may be one of a only few dominant exposure pathways for children 3-5 months old, but as children age their

consumption of food and water increases, and for children 12-23 months old, domestic soils may be one of many diverse exposure pathways.[259,262] As such, the observed difference between estimated risks and infection prevalence may be a product of high reinfection pressures and the presence of persistent infections.[258,259] There is evidence to suggest acquired immunity to *Giardia duodenalis* and *Shigella* spp. is possible, but it is not well characterized if such immunity may prevent infection, illness, or both.[253,263] The number of children who acquire immunity to infection by *Giardia duodenalis* or *Shigella* spp. would likely increase with age, but instead increasing infection prevalence with age was observed, suggesting immunity to infection by either pathogen is uncommon in low-income Maputo.

Our analysis is constrained by a number of important limitations. First, we did not collect site specific ingestion data, but instead relied on parameters from two plausible soil ingestion rate scenarios. Second, the dose-response models we used are derived from studies of healthy adults in the United States. Their applicability to young children in a LMIC is not clear as repeated infections may compromise the immune system resulting in greater susceptibility to infection, or conversely, acquired immunity due to endemic exposure.[253,263] We treated these input parameters as stochastic distributions to propagate this uncertainty. In addition, our analysis was limited to samples collected from three compound locations during the dry season. Analysis of other compound locations, or the same locations during the rainy season, may have resulted in a lower or higher prevalence and density of gene targets, but the widespread detection of gene targets at all three intra-compound locations suggests the soils we analyzed may be representative of domestic soils in this setting. Finally, *Giardia duodenalis* cysts are more persistent in the environment

than *E. coli*.[188,193] This may suggest that the proportion of viable *Giardia duodenalis* we used – which was a ratio of culturable *E. coli* to gene copies of *E. coli* – could underestimate cyst viability. In fact, given the model's sensitivity to the viability parameter, an underestimate of cyst viability would cause our results to underestimate *Giardia duodenalis* infection risks. However, bacteria are capable of re-growth and *E. coli* may be naturalized in soils[55,56], which also makes it possible our model overestimates cyst viability and infection risks from *Giardia duodenalis.* In addition, we tested for *Giardia duodenalis* assemblage B, but assemblage A is also infectious to humans[239] and subsequently our approach may have underestimated the infection risks posed by *Giardia duodenalis*.

In LMICs where the relationship between sanitation and health is complex, we offer evidence that children's ingestion of fecally contaminated soils results in a high risk of infection with *Giardia duodenalis* and *Shigella* spp.. Comparison with infection prevalence data from this setting, and the results of a sensitivity analysis, suggest that interventions to reduce children's ingestion of fecally contaminated soils are needed in this setting (e.g. contact control or source control). Similar stochastic QMRA models that use objective measures of enteric pathogen gene targets from the environment in LMICs may offer improved insight into local infection risks and inform locally relevant intervention strategies.

CHAPTER 8. CONCLUSION

Here we offered the first peer reviewed evidence that open defecation is common in a major American city and may pose risks to public health. As a replacement for the current approach employed by many US cities – criminalization of open defecation through anti-nuisance laws[139] – new policies are needed to increase access to sanitation by people experiencing homelessness. Such interventions may include new construction of public sanitation, employing bathroom attendants to increase accessibility, partnering with local businesses to increase access to publicly available restrooms, and expansion of housing programs such as Housing First[264].

As part of the MapSan trial in Maputo, Mozambique we evaluated the intervention's impact on pit emptying and the spread of enteric pathogens into latrine entrance soils. We found a positive impact of the intervention on the likelihood of hygienic emptying and a protective effect against the spread of some pathogenic bacteria into latrine entrance soils. However, 24-months following the intervention some intervention compounds (27%) reported being unsure or that they had emptied unhygienically in the previous year, and we detected at least one enteric pathogen in 84% of latrine entrance soils. More comprehensive WASH interventions may be needed in similar settings to dramatically limit the spread of fecal contamination through site specific pathways.

Sewage surveillance is an increasingly used approach with the potential to rapidly provide stakeholders with data on community health. Our analysis of matched stools and fecal sludges from low-income urban Maputo, Mozambique indicates that fecal sludge from on-site sanitation systems may be useful for pathogen surveillance. Such a tool may

be helpful to characterize the pathogens circulating in a community and inform comprehensive packages of WASH interventions that are tailored to address the local exposure landscape.

8.1 FUTURE RESEARCH DIRECTIONS

8.1.1 Water and Sanitation Access in the US

Though no city wants to be known for the prevalence of human feces strewn about on its sidewalks, open defecation is common in Atlanta and likely in other US cities. As the number of people experiencing homelessness in unsheltered locations continues to rise, public WASH facilities remain scarce, which may increase the likelihood that people resort to open defecation. Future progress towards universal safely managed sanitation in the urban US requires careful consideration that increased awareness may result in meaningful change, or negative consequences such as increased criminalization.

We have shown that the current data provided by the US to the WHO/UNICEF JMP vastly underestimates the total number of people in the urban US without sustained access to a flush toilet. Future progress likely will require human rights-based policy changes. Inter-disciplinary work between WASH researchers and other stakeholders – such as my work with Dr. Liz Frye from the Street Medicine Institute[62] – is needed to advocate for consistent access to water and sanitation as a part of broader housing initiatives.

8.1.2 Health impact of sanitation in LMICs

The results from the WASH-B[38,39], SHINE[40], and MapSan[43] trials indicate that on-site sanitation improvements may not radically improve children's health in LMICs. In

response, the field has called for transformative WASH which offers a comprehensive package of interventions specific to the local exposure landscape.[57] Research is needed to define what interventions can be considered comprehensive or "transformative".

First, if on-site improvements cannot reliably achieve improved health outcomes, then the question arises at what scale are interventions necessary to yield health gains. In LMICs, the "shit flow diagram", developed by the World Bank, represents the flows of excreta through the fecal waste disposal chain at a city level (Figure 7). The green arrows indicate excreta that is safely managed while the red arrows indicate excreta that is not safely managed. Combining quantitative measures of enteric pathogens at each point in the fecal waste disposal chain would enable a similar approach for pathogen flows at a city level. Such an approach may inform at what scale of interventions are necessary to reduce pathogen spread into the environment, which may inform future intervention strategies.

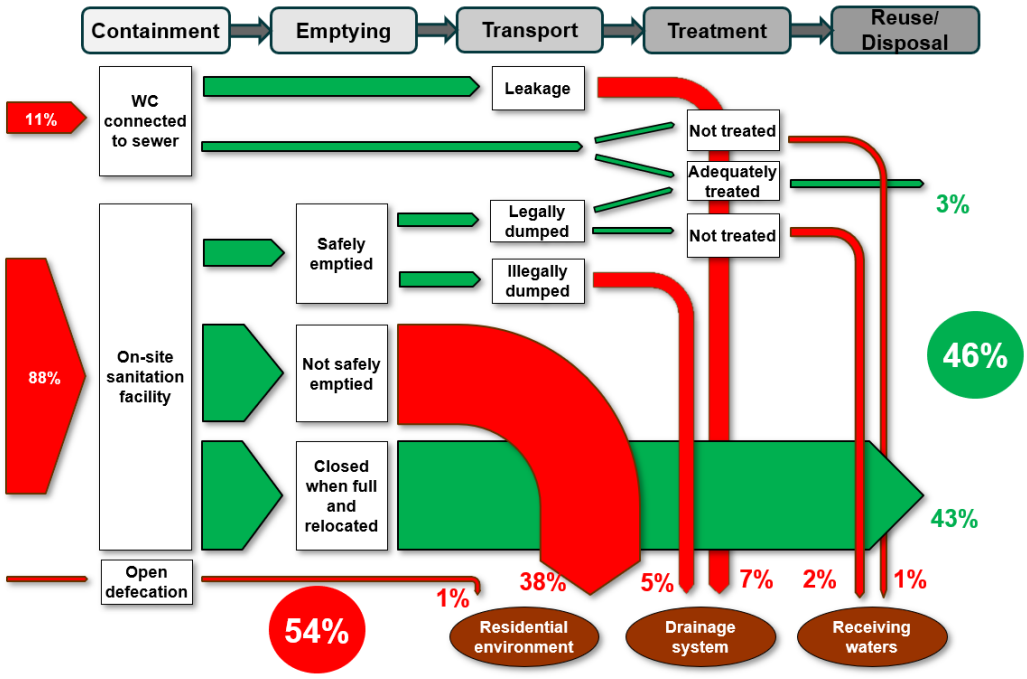

Figure 7. The Shit Flow Diagram (SFD) for Maputo. Mozambique. Available at: https://www.susana.org/_resources/documents/default/3-2272-7-1435310214.pdf)

Second, further work is needed to assess the differences in health outcomes between the urban poor, such as those in the MapSan cohort, and households of higher socioeconomic status. These studies may help explain what reductions in exposures, that may result from improved housing, diet or other lifestyle factors that could be expected from a higher socioeconomic status, are needed to improve children's health outcomes in LMICs.

8.1.3 Pathogen and AMR Surveillance

As a proof-of-concept we have demonstrated the potential to use fecal sludges in pathogen surveillance in LMICs. In cities such as Maputo, where less than 10% of the population is connected to a sewer, a logical next step is to investigate whether the pathogen and anti-microbial resistance signal observed in wastewater is substantially different than in fecal

sludges. Demonstrating such a difference would have serious implications for the Global Sewage Surveillance Project, which intends for a global body, such as the World Health Organization, to take over the current surveillance initiatives.[79,178] Considering the high burden of disease and widespread fecal contamination observed during the MapSan trial, using sewage and not sludges for pathogen and AMR surveillance in cities such as Maputo likely underestimates the burden of infection and the resistome.

In addition, widespread use of fecal sludges in health surveillance first requires standardized methods for representative sampling. Studies are needed to explore the concentration of pathogens and AMR genes in sludges, and their association with sludge solids content and abundance relative to the microbial community. These data would inform what sample volumes may be considered representative, and the variation between samples could be compared to assess if composite samples would be more representative than individual samples. Further, longitudinal studies are needed to assess the sensitivity of pathogen and AMR signals in sludges to infection prevalence and incidence over time. Refinements of these methods may allow for sludges to serve as an early warning of disease outbreaks and to be used for health impact assessment in WASH intervention trials.

In fact, fecal sludges, or other environmental matrices such as soils, offer WASH researchers the opportunity to "fail faster". Meaning that, these matrices present cheaper and faster methods to identify which interventions are most likely to improve health outcomes. The WASH-B, SHINE, and MapSan trials cost millions of dollars and required years of effort by hundreds of individuals. Less invasive environmental sampling, which may require fewer samples than stool-based studies to indicate whether a health outcome is probable, could form the basis for multiple pilot studies of different sanitation

interventions. Then larger studies could be designed around the interventions with the greatest reduction in environmental fecal contamination.

8.1.4 QMRA

Originally developed using culture-based methods, the field of QMRA has grown substantially since the 1980s with the adoption of new molecular based techniques.[89] Although, due to the limitations of culture and molecular approaches, additional methodological triangulation is needed to improve risk estimates. Harmonization of these approaches would then be helpful for comparison across studies. In fact, the integration of QMRA and epidemiology, which can estimate the same public health measures but produce widely different results, presents an opportunity to investigate and improve of each method.

In LMICs, applying QMRA to the fecal-oral transmission pathways represented by the F-diagram[23] may help characterize the local exposure landscape. While similar approaches have been conducted[265], they have relied on fecal indicator bacteria, and quantitative measures of enteric pathogens would better characterize infection risks from each pathway. A QMRA approach might inform the dominant infection pathways in a setting and inform strategies to reduce infection risks.

APPENDIX A. SUPPLEMENTAL MATERIALS TO CHAPTER 4

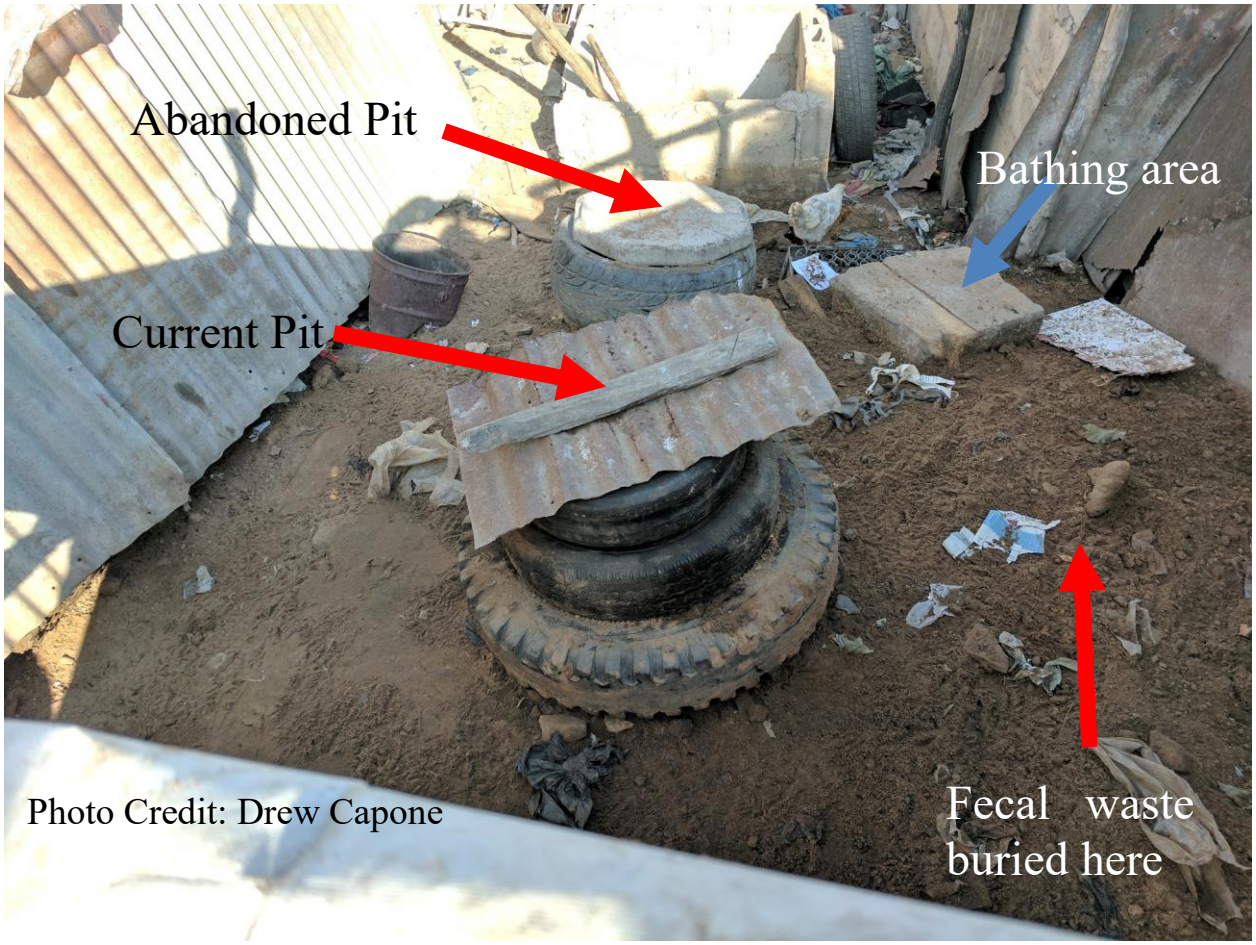

Figure A1. Bathroom in low-income urban Maputo, Mozambique

Figure A3. Community sanitation block

Figure A2. Shared latrine

Figure A4. Inside of a community sanitation block

A.1 Detailed description of the sanitation intervention

WSUP initially built 250 shared toilets and 50 community sanitation blocks, but due to the depreciation of the Metical (Mozambican currency) in 2016-2017, WSUP built 150 additional shared toilets in the project area. Shared latrines became the property of the residents and included a toilet, superstructure, septic tank, and a lined infiltration pit. Community sanitation blocks officially remained the property of the municipality and included the same infrastructure as a shared latrine, but contained multiple toilets (one toilet per twenty people), a new piped water connection with a water storage tank, sink pedestal for handwashing (no running water but the drain was connected to the septic tank), rainwater harvesting tank, cement laundry basin, and community sanitation blocks with ≥60 residents received a urinal on an external wall of the structure which drained to the septic tank. Compound residents that received community sanitation blocks formed sanitation management committees, which were responsible for maintaining the sanitation infrastructure. The septic tanks in the shared latrines and community sanitation blocks were sized according to the number of users and were designed to be emptied every two years (assuming 40 liters accumulation per person per year). All intervention septic tanks contained an access port for hygienic emptying, but the ports were sealed shut and did not enable easy visual inspection of fecal sludge levels.

A.2 Method for identifying non-MapSan trial respondent.

In recognition that the caregivers of children enrolled in the MapSan trial (R1) represented a relatively homogenous group (predominantly women of reproductive age) a second compound respondent was included in the survey sample (R2). To select R2, enumerators

identified the third household on the right of the entrance to the compound (providing this was not the household of R1). R2 could be any gender or age, so long as they were over 18 and had lived on the compound for more than 30 days. If there was no one available in the identified household, or the respondent refused to participate, the enumerator would identify the next third house on the right.

A.3 Details regarding compounds that did not meet eligibility requirements

We deemed 48 control respondents from 30 control compounds ineligible for the study as the intervention latrine infrastructure was present. We deemed 15 intervention respondents from nine intervention compounds ineligible due to the absence of the intervention latrine infrastructure at the intervention compounds.

Table A1. Responses to whether WSUP visited the respondent and discussed pit-emptying (priority respondent)

	Priority Respondent	
Response	Control	Intervention
Pit-emptying was ever discussed at a visit by WSUP	12% (30/247)	27% (74/270)
Pit-emptying was never discussed at a visit by WSUP	13% (31/247)	23% (63/270)
Unsure	75% (186/247)	49% (133/270)

Table A2. Water access and availability

	Control				Intervention	
Water Source	MapSan respondent	non-MapSan respondent	Pour flush sanitation	Not pour flush sanitation	MapSan respondent	non-MapSan respondent
Tap in the home	24% (43/179)	15% (30/199)	19% (27/140)	19% (46/238)	34% (60/176)	24% (54/223)
Tap outside the home	36% (65/179)	40% (79/199)	50% (70/140)	31% (74/238)	40% (70/176)	39% (87/223)
Neighbor's tap	37% (66/179)	43% (85/199)	31% (43/140)	45% (108/238)	25% (44/176)	35% (77/223)
Public tap	2.8% (5/179)	2.5% (5/199)	0% (0/140)	4.2% (10/238)	0.57% (1/176)	2.2% (5/223)
Protected spring	0% (0/179)	0% (0/199)	0% (0/140)	0% (0/238)	0.57% (1/176)	0% (0/223)
Water availability						
<1 hour	0% (0/179)	0% (0/199)	0% (0/140)	0% (0/238)	0% (0/176)	0% (0/223)
1-3 hours	15% (26/179)	15% (29/199)	12% (17/140)	16% (38/238)	15% (27/176)	14% (31/223)
4-6 hours	36% (65/179)	37% (74/199)	38% (53/140)	36% (86/238)	39% (69/176)	43% (95/223)
7-8 hours	33% (59/179)	36% (72/199)	34% (48/140)	35% (83/238)	32% (57/176)	29% (64/223)
> 8 hours	16% (29/179)	12% (24/199)	16% (22/140)	13% (31/238)	13% (23/176)	15% (33/223)

Table A3. How will residents decide the sanitation system requires emptying (all respondents)

Next time a sanitation structure is emptied, how will people in the compound determine the sanitation structure needs to be emptied?	Control latrine (with or without a slab)	Control with pour-flush technology	All control compounds	Intervention compounds
Smell	7.0% (23/327)	22% (40/180)	12% (63/507)	40% (215/542)
Visual inspection of fecal sludge level	38% (124/327)	32% (58/180)	36% (182/507)	19% (101/542)
A certain amount of time has passed	2.1% (7/327)	14% (25/180)	6.3% (32/507)	8.3% (45/542)
The sanitation structure is overflowing	6.1% (20/327)	18% (33/180)	10% (53/507)	19% (102/542)
The sanitation structure is damaged	0% (0/327)	0% (0/180)	0% (0/507)	55% (3/542)
Other	31% (1/327)	2.8% (5/180)	1.2% (6/507)	55% (3/542)
Unsure	46% (152/327)	11% (19/180)	34% (171/507)	13% (73/542)

Table A4. Intentions for future emptying (all respondents)

Reported intention for next time the sanitation system requires emptying	Control latrine (with or without a slab)	Control with pour-flush technology	All control compounds	Intervention compounds
Intend to cover the current pit and open a new pit in the compound	45% (140/327)	5.5% (10/180)	30% (150/507)	0% (2/542)
Intend to cover the current pit and use neighbor's latrine	0% (0/327)	0% (0/180)	0% (0/507)	0% (1/542)
Intend to empty by household member	6.7% (22/327)	7.2% (13/180)	7% (35/507)	1% (6/542)
Intend to empty by community member	34% (99/327)	22% (40/180)	27% (139/507)	17% (94/542)
Intend to empty by a business or non-governmental organization	3.7% (12/327)	43% (77/180)	18% (89/507)	58% (315/542)
Intend to replace the entire sanitation system	2.1% (7/327)	0% (0/180)	1% (7/507)	0% (0/542)
Unsure	14 (47/327)	22% (40/180)	17% (87/507)	23% (124/542)

A.4 Detailed description of fecal sludge transport

We asked survey respondents where their fecal sludge was disposed last time they emptied their sanitation system. As survey respondents had only witnessed pit-emptiers while they were on-site, they were likely able to recall if their fecal sludge was dumped or buried in or near their compound. However, if fecal sludge was transported away by a truck or tractor, they likely did not have certainty regarding the final disposal site for their fecal sludge.

In addition to compound interviews, we conducted structured in-depth interviews on FSM topics with the staff that took part in component two of the intervention and were equipped to provide pit-emptying services. We posed questions on the status and characteristics of the CBOs' emptying activities, their experience working with WSP, and their difficulties and successes as pit-emptiers in Maputo. Of the eight formal operators trained by WSUP as part of the JSDF funded intervention, we met with the four who had continued to service clients after the pilot period ended in 2015. We were unable to contact the four operators who had ceased servicing clients.

Due to the statements by the four pit-emptying organizations surveyed that they never illegal dumped fecal sludge, the illegality of dumping in Maputo, the close proximity of *Nhlamankulu* District to the WWTP (typically a 7-25 minute drive depending on intra-district location) and the potential for reputational risk to hygienic emptying companies who engage in illegal dumping, we inferred that respondents who stated their fecal sludge was transported away to an unknown location was indicative of hygienic transport to the local WWTP.

Table A5. Reasons for not stating a preference for hygienic emptying among those who stated a preference for future unhygienic emptying

	Intervention				Control			
Respondent	MapSan	Non-MapSan	Compound Leader	All	MapSan	Non-MapSan	Compound Leader	All
Cost	74% (32/43)	91% (29/32)	80% (20/25)	81% (81/100)	92% (54/59)	94% (60/64)	86% (44/51)	91% (158/174)
Access	23% (10/43)	6% (2/32)	20% (5/25)	17% (17/100)	5% (3/59)	5% (3/64)	12% (6/51)	7% (12/174)
Other*	2% (1/43)	3% (1/32)	0% (0/25)	2% (2/100)	3% (2/59)	2% (1/64)	2% (1/51)	2% (4/174)

*Other responses included unsure (n=4), “because all my neighbors do that [unhygienically empty]” (n=1), and “I don’t know anyone [who does hygienic emptying]” (n=1)

APPENDIX B. SUPPLEMENTAL MATERIALS TO CHAPTER 5

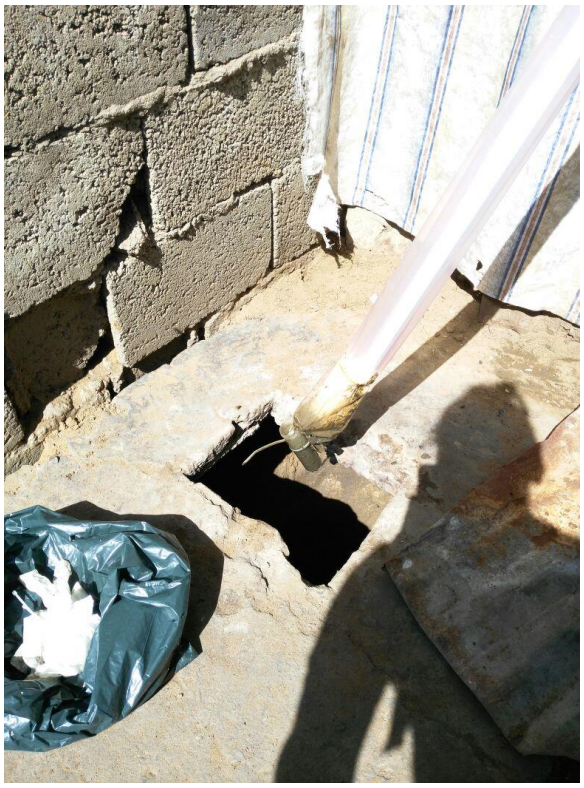

Figure B1. Sludge nabber

Figure B2. Modified Wheaton sub-surface sampler

B.1 Methodology for fecal sludge collection

To modify the sludge nabber, we first covered the sampler with disposable plastic tubing (Amazon.com, https://www.amazon.co.uk/Empire-Packaging%C2%AE-Layflat-Polythene-Tubing/dp/B01B2WT8SE/) (to limit direct contact with feces) and then used zip ties to fasten a 50mL centrifuge tube to the sampler. We inserted the sludge nabber down the drophole below the surface of the fecal sludge in pit latrines at one point (in the middle of the pit latrine). We submerged the sampler tube for 5 seconds and then withdrew it. We then capped and closed the sampler tube, sterilized the outside with bleach and placed it in

a cooler. Between pit latrine sampling events, we sprayed the sampler with bleach and allowed 15 minutes of contact time to sterilize it prior to drying it off with a fresh paper towel. Samples were stored on ice for transport, homogenized in the lab by combining the samples into one tube followed by manual shaking, aliquoted into 2-ml cryovials within 6 hours of collection and stored at -80^{o}C until analysis.

For septic tanks, we used a modified Wheaton sub-surface sampler I system (Fisher Scientific, Waltham, Massachusetts) to fill a 50 mL centrifuge tube. We laser cut extruded acrylic insert disks for the centrifuge tubes to be held in the sampler. We then inserted the centrifuge tube into the disks, clamped it firmly, and attached the suction top of the sampler to the tube, keeping the tube closer. At the septic tank, we opened the side access port (for emptying) and submerged the sampler into the solids layer at the bottom of the septic tank (generally the bottom 1-2 feet of the tank). We then twisted the suction arm to open the sampler for 5 seconds and closed it firmly before lifting the sampler out of the tank. We then removed the tube and sterilized the outside of the sampler and tube with bleach prior to drying it off with a fresh paper towel and placing the tube in a cooler. Samples were stored on ice for transport. To make sample aliquots we vortexed the 50 mL tubes for 10 seconds and pipetted 2 mL of fecal sludge into a 2 mL cryovial. Within 6 hours of collection samples were stored at -80^{o}C and remained frozen until analysis.

B.2 Methodology for nucleic acid extraction from fecal sludges and stools

We incubated 100 mg of each fecal sludge sample at 105^{o}C for 1 hour to determine moisture content. For compounds where we collected more than one child's stool prior to the fecal sludge sample, we *a priori* decided to analyze the oldest child's stool sample

because older children are more likely to have intra-compound exposures and defecate into the latrine compared to younger children.[78]

We vortexed fecal sludge samples (in 2 mL cryovials) for 3 seconds, then pipetted 100 μL of watery sludge or 100 mg of thick sludge or stool into a Bertin SK-38 (Bertin Corp, Rockville, MD) bead beating tube with 1 mL of Qiagen Buffer ASL (Qiagen, Hilden, Germany) and phage MS2 as an extraction control. We vortexed bead beating tubes for 5 minutes, incubated at room temperature for 15 minutes and then centrifuged at 14,000 rpm for 2 minutes. We proceeded with extraction following the manufacturer's protocol for the QIAamp 96 Virus QIAcube HT Kit, which we automated on the QIAcube (Qiagen, Hilden, Germany).

B.3 Custom TaqMan Array Card (TAC)

We purchased custom TACs produced by Thermo Fisher Scientific (Waltham, MA). TAC is a 384-well array card with 8 ports for loading samples and each well contains dried-down primers and hydrolysis probes for the detection of defined targets.

For analysis, we mixed 50 μL of total nucleic acid template with 50 μL of qScript XLT 1-Step RT-qPCR ToughMix (Quantabio, Beverly, MA), then filled ports 2-7 with the combined 100 μL. In total we tested 6 samples per card, using the first port as a negative control and the last port as a positive control, for which we used individual aliquots of our combined positive control material (gene targets inserted into plasmids) (IDT, Coralville, IA). Positive control material was constructed according to the method of Kodani *et al*. 2012.[266]

Following the manufacturer's instructions, we centrifuged each card twice at 1,200 rpm for one minute, sealed the card, trimmed the loading ports, and loaded the card into a QuantStudio 7 (Thermo Fisher Scientific, Waltham, MA). To perform reverse transcriptase quantitative PCR we used the following cycling conditions with a 1˚C/s ramp rate between all steps: 45˚C for 10 minutes, 94˚C for 10 minutes, and then 45 cycles of 94˚C for 30 seconds and 60˚C for 1 minute.

To compare our TAC's performance, we tested a subset of stool and fecal sludge samples using the Luminex Gastrointestinal Pathogen Panel[3,4] (Luminex, Austin, TX) – a FDA cleared assay – according to manufacturer's instructions.

Table B1. Comparison of GPP and TAC

Fecal Sludge Comparison (n = 26)				
Target	GPP Positive & TAC Positive	GPP Positive & TAC Negative	GPP Negative & TAC Positive	GPP Negative & TAC Negative
Adenovirus 40/41	2	1	11	12
Norovirus GI/GII	4	0	10	12
Rotavirus A	0	0	2	24
C. difficile toxin A/B	0	2	2	22
C. jejuni	1	0	0	25
ETEC LT/ST	12	0	4	10
STEC stx1/stx2	0	0	4	22
Shigella	17	3	3	3
Vibrio cholerae	0	0	0	26
Yersinia enterocolitica	0	0	0	26
Cryptosporidium	0	0	8	18
Entamoeba histolytica	1	0	4	21
Giardia spp.	22	0	0	4
Percentage	17%	1.8%	14%	67%
Stool comparison (n = 91)				
Target	GPP Positive & TAC Positive	GPP Positive & TAC Negative	GPP Negative & TAC Positive	GPP Negative & TAC Negative
Adenovirus 40/41	2	0	2	87
Norovirus GI/GII	5	1	6	79
Rotavirus A	0	0	1	90
C. difficile toxin A/B	1	1	2	87
C. jejuni	3	1	2	85
ETEC LT/ST	19	6	13	53
STEC stx1/stx2	5	2	1	83
Shigella	42	3	5	41
Vibrio cholerae	0	0	0	91
Yersinia enterocolitica	0	0	0	91
Cryptosporidium	1	1	8	81
Entamoeba histolytica	1	1	0	89
Giardia spp.	53	1	7	30
Percentage	11%	1.4%	4.0%	83%

Table B1. Assays used on the custom TAC

Target	Assay reference
Bacteria	
Campylobacter coli	Cunningham, S. A.; Sloan, L. M.; Nyre, L. M.; Vetter, E. A.; Mandrekar, J.; Patel, R. Three-Hour Molecular Detection of Campylobacter, Salmonella, Yersinia, and Shigella Species in Feces with Accuracy as High as That of Culture. *J. Clin. Microbiol.* **2010**, *48* (8), 2929–2933.
Campylobacter jejuni	Cunningham, S. A.; Sloan, L. M.; Nyre, L. M.; Vetter, E. A.; Mandrekar, J.; Patel, R. Three-Hour Molecular Detection of Campylobacter, Salmonella, Yersinia, and Shigella Species in Feces with Accuracy as High as That of Culture. *J. Clin. Microbiol.* **2010**, *48* (8), 2929–2933.
Clostridium difficile (*tcdA*)	Houser, B. A.; Hattel, A. L.; Jayarao, B. M. Real-Time Multiplex Polymerase Chain Reaction Assay for Rapid Detection of Clostridium Difficile Toxin-Encoding Strains. *Foodborne Pathog. Dis.* **2010**, *7* (6), 719–726.
Clostridium difficile (*tcdB*)	Houser, B. A.; Hattel, A. L.; Jayarao, B. M. Real-Time Multiplex Polymerase Chain Reaction Assay for Rapid Detection of Clostridium Difficile Toxin-Encoding Strains. *Foodborne Pathog. Dis.* **2010**, *7* (6), 719–726.
E. coli / Shigella (*ipaH* gene)	Thiem, V. D.; Sethabutr, O.; Seidlein, L. von; Tung, T. Van; Canh, D. G.; Chien, B. T.; Tho, L. H.; Lee, H.; Houng, H.-S.; Hale, T. L.; et al. Detection of Shigella by a PCR Assay Targeting the IpaH Gene Suggests Increased Prevalence of Shigellosis in Nha Trang, Vietnam. *J. Clin. Microbiol.* **2004**, *42* (5), 2031–2035.
EAEC (*aaiC* gene)	Liu, J.; Gratz, J.; Amour, C.; Kibiki, G.; Becker, S.; Janaki, L.; Verweij, J. J.; Taniuchi, M.; Sobuz, S. U.; Haque, R.; et al. A Laboratory-Developed Taqman Array Card for Simultaneous Detection of 19 Enteropathogens. *J. Clin. Microbiol.* **2013**, *51* (2), 472–480.
EAEC (*aatA* gene)	Boisen, N.; Struve, C.; Scheutz, F.; Krogfelt, K. A.; Nataro, J. P. New Adhesin of Enteroaggregative Escherichia Coli Related to the Afa/Dr/AAF Family. *Infect. Immun.* **2008**, *76* (7), 3281–3292.
EPEC (*bfpA* gene)	Liu, J.; Gratz, J.; Amour, C.; Kibiki, G.; Becker, S.; Janaki, L.; Verweij, J. J.; Taniuchi, M.; Sobuz, S. U.; Haque, R.; et al. A Laboratory-Developed Taqman Array Card for Simultaneous Detection of 19 Enteropathogens. *J. Clin. Microbiol.* **2013**, *51* (2), 472–480.
EPEC (*eae* gene)	Liu, J.; Gratz, J.; Amour, C.; Kibiki, G.; Becker, S.; Janaki, L.; Verweij, J. J.; Taniuchi, M.; Sobuz, S. U.; Haque, R.; et al. A Laboratory-Developed Taqman Array Card for Simultaneous Detection of 19 Enteropathogens. *J. Clin. Microbiol.* **2013**, *51* (2), 472–480.
ETEC-LT	Hidaka, A.; Hokyo, T.; Arikawa, K.; Fujihara, S.; Ogasawara, J.; Hase, A.; Hara-Kudo, Y.; Nishikawa, Y. Multiplex Real-Time PCR for Exhaustive Detection of Diarrhoeagenic *Escherichia Coli*. *J. Appl. Microbiol.* **2009**, *106* (2), 410–420.
ETEC-ST	Liu, J.; Gratz, J.; Amour, C.; Kibiki, G.; Becker, S.; Janaki, L.; Verweij, J. J.; Taniuchi, M.; Sobuz, S. U.; Haque, R.; et al. A Laboratory-Developed Taqman Array Card for Simultaneous Detection of 19 Enteropathogens. *J. Clin. Microbiol.* **2013**, *51* (2), 472–480.

Table B2 continued.

Salmonella	Liu, J.; Gratz, J.; Amour, C.; Kibiki, G.; Becker, S.; Janaki, L.; Verweij, J. J.; Taniuchi, M.; Sobuz, S. U.; Haque, R.; et al. A Laboratory-Developed Taqman Array Card for Simultaneous Detection of 19 Enteropathogens. *J. Clin. Microbiol.* **2013**, *51* (2), 472–480.
Shiga-like toxin 1 (*stx1*)	Liu, J.; Gratz, J.; Amour, C.; Kibiki, G.; Becker, S.; Janaki, L.; Verweij, J. J.; Taniuchi, M.; Sobuz, S. U.; Haque, R.; et al. A Laboratory-Developed Taqman Array Card for Simultaneous Detection of 19 Enteropathogens. *J. Clin. Microbiol.* **2013**, *51* (2), 472–480.
Shiga-like toxin 2 (*stx2*)	Hidaka, A.; Hokyo, T.; Arikawa, K.; Fujihara, S.; Ogasawara, J.; Hase, A.; Hara-Kudo, Y.; Nishikawa, Y. Multiplex Real-Time PCR for Exhaustive Detection of Diarrhoeagenic *Escherichia Coli*. *J. Appl. Microbiol.* **2009**, *106* (2), 410–420.
Vibrio cholerae	Liu, J.; Gratz, J.; Amour, C.; Kibiki, G.; Becker, S.; Janaki, L.; Verweij, J. J.; Taniuchi, M.; Sobuz, S. U.; Haque, R.; et al. A Laboratory-Developed Taqman Array Card for Simultaneous Detection of 19 Enteropathogens. *J. Clin. Microbiol.* **2013**, *51* (2), 472–480.
Yersinia spp.	Liu, J.; Gratz, J.; Maro, A.; Kumburu, H.; Kibiki, G.; Taniuchi, M.; Howlader, A. M.; Sobuz, S. U.; Haque, R.; Talukder, K. A.; et al. Simultaneous Detection of Six Diarrhea-Causing Bacterial Pathogens with an In-House PCR-Luminex Assay. *J. Clin. Microbiol.* **2012**, *50* (1), 98–103.
Viruses	
Adenovirus 40/41	Jothikumar, N.; Cromeans, T. L.; Hill, V. R.; Lu, X.; Sobsey, M. D.; Erdman, D. D. Quantitative Real-Time PCR Assays for Detection of Human Adenoviruses and Identification of Serotypes 40 and 41. *Appl. Environ. Microbiol.* **2005**, *71* (6), 3131–3136.
Astrovirus	Liu, J.; Kibiki, G.; Maro, V.; Maro, A.; Kumburu, H.; Swai, N.; Taniuchi, M.; Gratz, J.; Toney, D.; Kang, G.; et al. Multiplex Reverse Transcription PCR Luminex Assay for Detection and Quantitation of Viral Agents of Gastroenteritis. *J. Clin. Virol.* **2011**, *50* (4), 308–313.
Norovirus GI	Jothikumar, N.; Lowther, J. A.; Henshilwood, K.; Lees, D. N.; Hill, V. R.; Vinjé, J. Rapid and Sensitive Detection of Noroviruses by Using TaqMan-Based One-Step Reverse Transcription-PCR Assays and Application to Naturally Contaminated Shellfish Samples. *Appl. Environ. Microbiol.* **2005**, *71* (4), 1870–1875.
Norovirus GII	Kageyama, T.; Kojima, S.; Shinohara, M.; Uchida, K.; Fukushi, S.; Hoshino, F. B.; Takeda, N.; Katayama, K. Broadly Reactive and Highly Sensitive Assay for Norwalk-like Viruses Based on Real-Time Quantitative Reverse Transcription-PCR. *J. Clin. Microbiol.* **2003**, *41* (4), 1548–1557.
Rotavirus A	Jothikumar, N.; Kang, G.; Hill, V. R. Broadly Reactive TaqMan® Assay for Real-Time RT-PCR Detection of Rotavirus in Clinical and Environmental Samples. *J. Virol. Methods* **2009**, *155* (2), 126–131.

Table B3 continued

Sapovirus I/II/IV	Liu, J.; Gratz, J.; Amour, C.; Kibiki, G.; Becker, S.; Janaki, L.; Verweij, J. J.; Taniuchi, M.; Sobuz, S. U.; Haque, R.; et al. A Laboratory-Developed Taqman Array Card for Simultaneous Detection of 19 Enteropathogens. *J. Clin. Microbiol.* **2013**, *51* (2), 472–480.
Sapovirus V	Liu, J.; Gratz, J.; Amour, C.; Kibiki, G.; Becker, S.; Janaki, L.; Verweij, J. J.; Taniuchi, M.; Sobuz, S. U.; Haque, R.; et al. A Laboratory-Developed Taqman Array Card for Simultaneous Detection of 19 Enteropathogens. *J. Clin. Microbiol.* **2013**, *51* (2), 472–480.
Protozoa	
Cryptosporidium parvum	Jothikumar, N.; da Silva, A. J.; Moura, I.; Qvarnstrom, Y.; Hill, V. R. Detection and Differentiation of Cryptosporidium Hominis and Cryptosporidium Parvum by Dual TaqMan Assays. *J. Med. Microbiol.* **2008**, *57* (9), 1099–1105.
Entamoeba histolytica	Verweij, J. J.; Blangé, R. A.; Templeton, K.; Schinkel, J.; Brienen, E. A. T.; van Rooyen, M. A. A.; van Lieshout, L.; Polderman, A. M. Simultaneous Detection of Entamoeba Histolytica, Giardia Lamblia, and Cryptosporidium Parvum in Fecal Samples by Using Multiplex Real-Time PCR. *J. Clin. Microbiol.* **2004**, *42* (3), 1220–1223.
Giardia duodenalis	Verweij, J. J.; Blangé, R. A.; Templeton, K.; Schinkel, J.; Brienen, E. A. T.; van Rooyen, M. A. A.; van Lieshout, L.; Polderman, A. M. Simultaneous Detection of Entamoeba Histolytica, Giardia Lamblia, and Cryptosporidium Parvum in Fecal Samples by Using Multiplex Real-Time PCR. *J. Clin. Microbiol.* **2004**, *42* (3), 1220–1223.
Soil-transmitted helminths	
Ascaris lumbricoides	Wiria, A. E.; Prasetyani, M. A.; Hamid, F.; Wammes, L. J.; Lell, B.; Ariawan, I.; Uh, H. W.; Wibowo, H.; Djuardi, Y.; Wahyuni, S.; et al. Does Treatment of Intestinal Helminth Infections Influence Malaria? Background and Methodology of a Longitudinal Study of Clinical, Parasitological and Immunological Parameters in Nangapanda, Flores, Indonesia (ImmunoSPIN Study). *BMC Infect. Dis.* **2010**, *10* (1), 77.
Trichuris trichiuria	Pilotte, N.; Papaiakovou, M.; Grant, J. R.; Bierwert, L. A.; Llewellyn, S.; McCarthy, J. S.; Williams, S. A. Improved PCR-Based Detection of Soil Transmitted Helminth Infections Using a Next-Generation Sequencing Approach to Assay Design. *PLoS Negl. Trop. Dis.* **2016**, *10* (3), e0004578.

Table B4. Interpretation of gene targets on the TAC

Target	Gene Targeted	Interpretation
Bacteria		
Campylobacter coli	*cadF* gene	If either was detected, call as *Campylobacter coli/jejuni* positive
Campylobacter jejuni	*cadF gene*	
Clostridium difficile (*tcdA*)	*tcdA* gene	If either was detected, call as *Clostridium difficile* positive
Clostridium difficile (*tcdB)*	*tcdB gene*	
E. coli / Shigella (*ipaH*)	*ipaH* gene	If detected, call as *Shigella*/EIEC positive
EAEC (*aaiC*)	*aaiC* gene	If either was detected, call as EAEC positive
EAEC (*aatA*)	*aatA* gene	
EPEC (*bfpA*)	*bfpA* gene	If either was detected, call as EPEC positive
EPEC (*eae*)	*eae* gene	
ETEC-LT	*LT* gene	If either was detected, call as ETEC positive
ETEC-ST	*STh/STp*	
Salmonella spp.	*invA* gene	If detected, call as *Salmonella spp.* positive
Shiga-like toxin 1 (stx1)	*stx*$_1$ gene	If either was detected, call as STEC positive
Shiga-like toxin 2 (stx2)	*stx*$_2$ gene	
Vibrio cholerae	*toxR* gene	If detected, call as *Vibrio cholerae* positive
Yersinia spp.	*lysP* gene	If detected, call as *Yersinia spp.* positive
Viruses		
Adenovirus 40/41	*Fiber* gene	If detected, call as Adenovirus 40/41 positive
Astrovirus	*Capsid* gene	If detected, call as Astrovirus positive
Norovirus GI	*ORF1-ORF2* gene	If either was detected, call as Norovirus GI/GII positive
Norovirus GII	*ORF1-ORF2* gene	
Rotavirus A	*NSP3* gene	If detected, call as Rotavirus A positive
Sapovirus I/II/IV	*RdRp* gene	If either was detected, call as Sapovirus positive
Sapovirus V	*RdRp* gene	

Table B5 continued

Protozoa		
Cryptosporidium parvum	*18S*	If detected, call as *Cryptosporidium parvum* positive
Entamoeba histolytica	*18S*	If detected, call as *Entamoeba histolytica* positive
Giardia duodenalis	*18S*	If detected, call as *Giardia duodenalis* positive
Helminth		
Ascaris lumbricoides	*18S*	If detected, call as *Ascaris lumbricoides* positive
Trichuris trichiuria	*ITS1*	If detected, call as *Trichuris trichiuria* positive

B.4 Regression models

We analyzed data in R version 4.0.0 (R Foundation for Statistical Computing, Vienna, Austria). We fit separate models with the number of bacteria, viruses, protozoa, and helminths detected in the two matrices as the response variables. We used the base R function glm() with a Poisson (log) distribution to assess factors that may have impacted pathogen detection in stools and fecal sludges.

For model diagnostics, we assessed dispersion using the *dispersiontest* function from the *AER* package in R. All models were under-dispersed, so we retained the Poisson distribution.

We used the Simple Poverty Scorecard® Poverty-Assessment Tool for Mozambique (Mark Schreiner 2013) and data from the MapSan 24-month follow-up dataset to calculate wealth scores.

B.4.1 Pathogens detected in stools

For pathogens detected in stools, we *a priori* decided to adjust our models for variables with strong plausibility as risk factors for enteric infection. We included the full model to assess each exposure variable compared to the reference: children's age versus children 1-23 month's old, wealth score versus a one-quartile increase in wealth score, compound population versus a 10-person increase in compound population, and the type of on-site sanitation versus a pit latrine. We obtained data for all variables from the MapSan 24-month follow-up dataset. To account for the missing data (17/95 child's ages) we used multiple imputation with chained equations (mice package in R) to create 50 complete

datasets (m=50) containing 50 iterations (maxit=50), and predictive mean matching (meth= "pmm").

We developed directed acyclic graphs to represent our models. We fit models using the number of pathogenic bacteria (from 0 to 10), viruses (from 0 to 5), protozoa (from 0 to 3), or helminths (from 0 to 2) as the response variables.

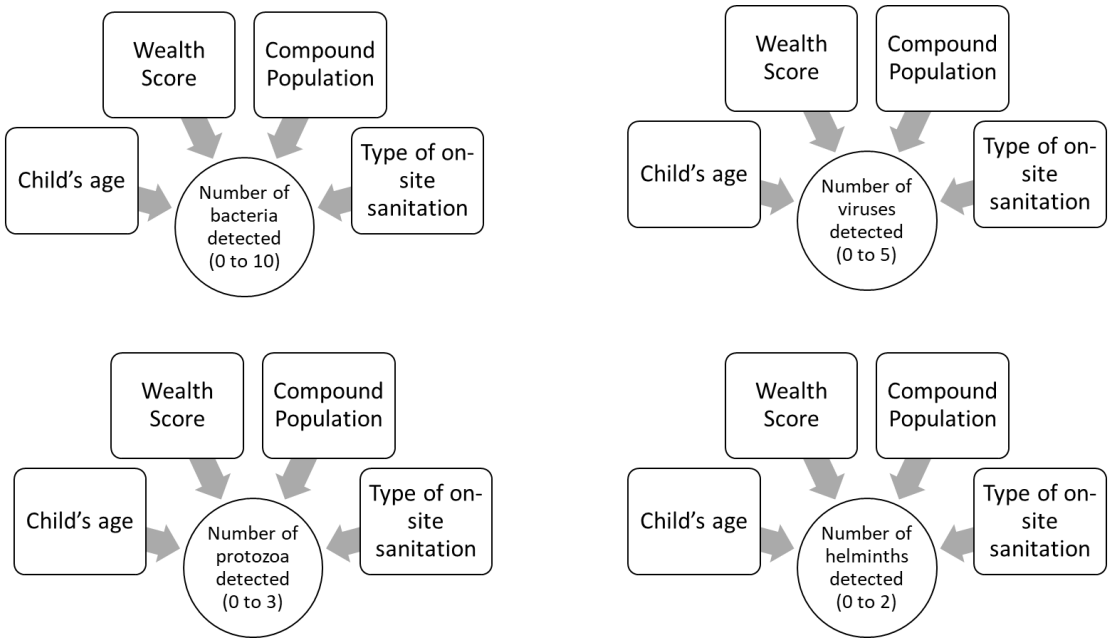

Figure B3. Directed acyclic graphs representing GLMs with the number pathogenic bacteria, viruses, protozoa, or STHs detected in stools as the dependent variable

B.4.2 Pathogens detected in fecal sludges

For pathogens detected in fecal sludges, we a priori decided to adjust our models for variables with strong plausibility as risk factors for enteric pathogen detection in fecal sludges. We included the full model to assess each exposure variable compared to the reference: children's age versus children 1-23 month's old, wealth score versus a one-quartile increase in wealth score, compound population versus a 10-person increase in

compound population, and the type of on-site sanitation versus a pit latrine. We obtained data for variables (type of on-site sanitation system, wealth score, compound population) from the MapSan 24-month follow-up dataset and our experimental data (fecal sludge solids content).

We developed directed acyclic graphs to represent our models. We fit models using the the number of pathogenic bacteria (from 0 to 10), viruses (from 0 to 5), protozoa (from 0 to 3), or helminths (from 0 to 2) as the dependent variables.

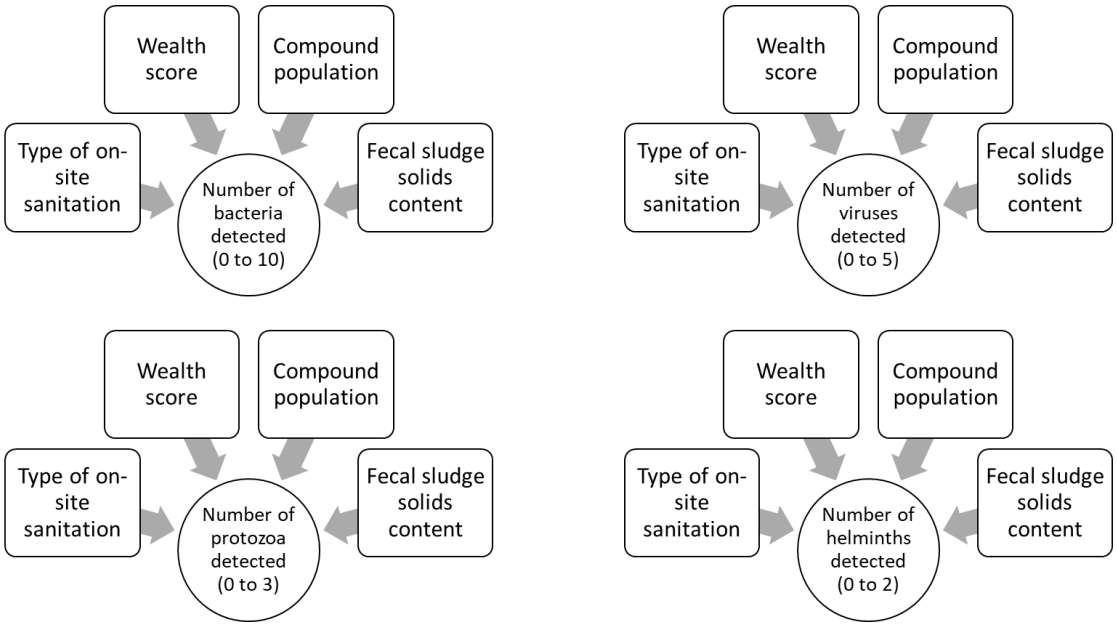

Figure B4. DAGs of sludge models

APPENDIX C. SUPPLEMENTAL MATERIALS TO CHAPTER 6

Table C1. Description of variables and their respective sources

	Variable description	**Data source**
Outcome Data		
Presence of bacteria/virus/protozoa/STH in latrine entrance soils	Binary detect/non-detect; 1/0	Experimental data
Number of bacteria/virus/protozoa/STH detected in latrine entrance soils	Integer; from 0 to N, where N is the number of targets on TAC for each pathogen type	Experimental data
Presence of individual pathogens on TAC	Binary detect/non-detect; 1/0	Experimental data
Covariates used in multivariate model selection		
Compound population	Continuous variable: transformed to represent a 10-person increase	Baseline and 24-month datasets
Wealth index	Quartile (1, 2, 3, or 4) derived from a continuous variable (from 0 to 1)	Baseline and 24-month datasets (Calculated using the Simple Poverty Scorecard® Poverty-Assessment Tool: Mozambique)
Visibly wet soil	Wet/dry; 1/0	Observed and recorded by enumerator at time of sampling
Average temperature in Fahrenheit during the day of and day before the soil sample was collected (e.g. 2-day average temperature)	Continuous variable, mean centered	Downloaded data from the National Oceanic and Atmospheric Administration's National Centers for Environmental Information

		(https://www.ncdc.noaa.gov/cdo-web/datatools/findstation)
Baseline and 24-month sanitation infrastructure	Factor; Pit latrine (without slab), pit latrine (with slab), intervention pour-flush toilet, non-intervention pour flush toilet, or unusable latrine (e.g. used neighbor's latrine or reported open defecation)	Baseline and 24-month datasets In addition, we reviewed illustrative photographs of sanitation infrastructure to confirm the sanitation infrastructure present
Dog(s) present	Binary, present / not present; 1/0	Baseline and 24-month datasets
Chicken(s)/duck(s) present	Binary, present / not present; 1/0	Baseline and 24-month datasets
Cat(s) present	Binary, present / not present; 1/0	Baseline and 24-month datasets
Visible feces in the compound (human or animal)	Binary, present / not present; 1/0	Baseline and 24-month datasets
Full pit	Binary: full / not full; 1/0	24-month dataset

C.1 Compound cross-over at the 24-month phase

To be included in the sensitivity analysis at the 24-month phase intervention compounds were required to have the intervention infrastructure and control compounds were not allowed to possess the intervention infrastructure. Due to the length of follow-up, some intervention compounds may have removed the intervention infrastructure to make room for housing construction or other construction needs. Or it is possible that some intervention compounds never received the intervention. In addition, due to the depreciation of the Mozambican Metical against the dollar from 2015-2016, WSUP had excess funds and delivered additional shared latrines in 2016 and 2017. Some controls compounds received these shared latrines after baseline, but before the 24-month follow-up.

Table C2. Sanitation infrastructure at baseline and the 24-month phase

Sanitation at baseline		
Sanitation infrastructure	Control	Intervention
Used neighbor's latrine	2.1% (1/47)	2.3% (1/44)
Open defecation	4.3% (2/47)	6.8% (3/44)
Pit latrine (without slab)	26% (12/47)	64% (28/44)
Pit latrine (with slab)	64% (30/47)	25% (11/44)
Non-intervention pour-flush toilet	4.3% (2/47)	2.3% (1/44)
Sanitation at 24-month phase		
Sanitation infrastructure	Control	Intervention
Used neighbor's latrine	0% (0/44)	0% (0/44)
Open defecation	0% (0/44)	0% (0/44)
Pit latrine (without slab)	30% (13/44)	2.3% (1/44)
Pit latrine (with slab)	32% (14/44)	9.1% (4/44)
Intervention pour-flush toilet	14% (6/44)	89% (39/44)
Non-intervention pour-flush toilet	25% (11/44)	0% (0/44)

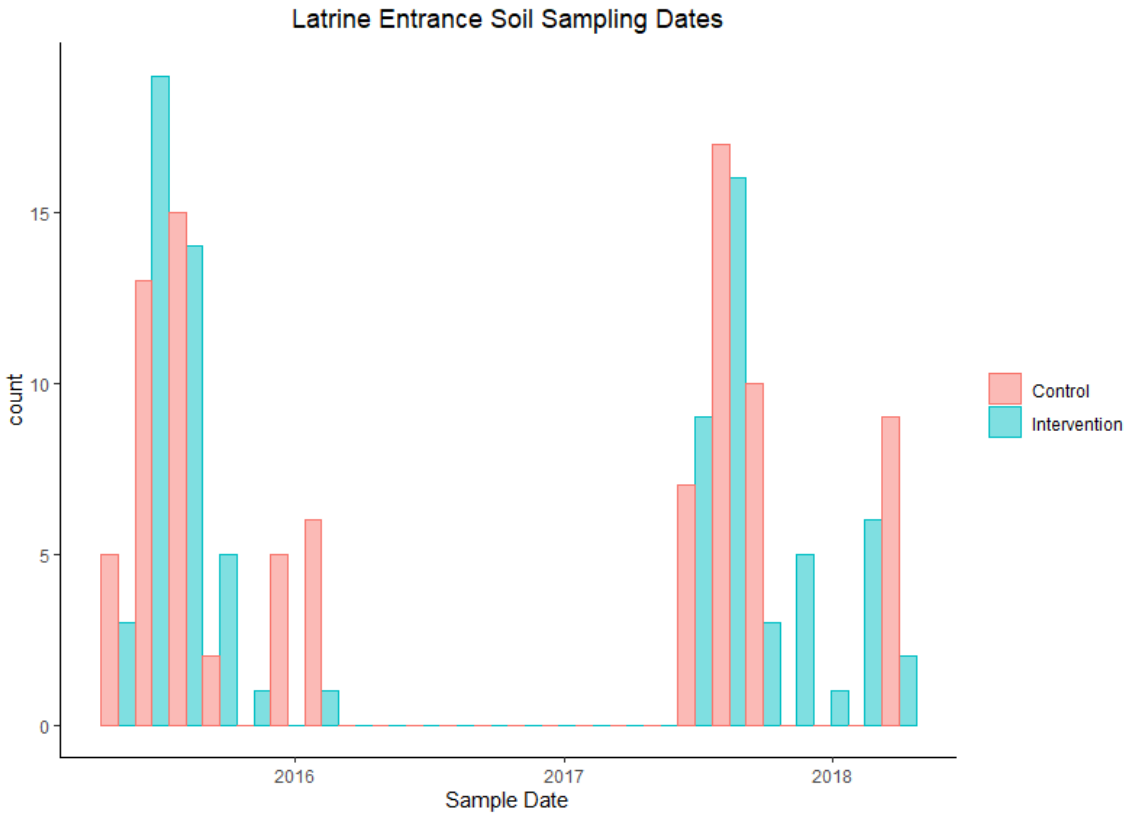

Figure C1. Histogram of dates that latrine entrance soils were collected

Table C3. Socio-demographic characteristics at baseline and 24-month follow-up

Phase		Variable	Mean (sd)	Median	range
Baseline	Control	Wealth index (0-1)	0.47 (0.09)	0.46	0.33, 0.78
	Intervention		0.46 (0.09)	0.45	0.28, 0.67
	Control	Compound population	15 (7.1)	13	6, 31
	Intervention		18 (7.6)	16	6, 34
24-month	Control	Wealth index (0-1)	0.44 (0.12)	0.47	0.13, 0.70
	Intervention		0.40 (0.08)	0.38	0.25, 0.67
	Control	Compound population	14 (7.4)	12	3, 31
	Intervention		15 (7.8)	13	6, 38

Table C4. DID analysis excluding cross-over compounds

Prevalence					
	Baseline Prevalence	24-month Prevalence		Unadjusted BL-24M DID estimate	Adjusted BL-24M DID estimate
Any pathogenic bacteria					
control	0.64 (30/47)	0.79 (30/38)			
intervention	0.80 (35/44)	0.67 (26/39)		0.67 (0.45, 1.0)	**0.66 (0.44, 0.98)**
Any pathogenic virus					
control	0.51 (24/47)	0.47 (18/38)			
intervention	0.39 (17/44)	0.36 (14/39)		1.0 (0.49, 2.0)	0.99 (0.49, 2.0)
Any pathogenic protozoa					
control	0.47 (22/47)	0.37 (14/38)			
intervention	0.41 (18/44)	0.36 (14/39)		1.1 (0.54, 2.2)	1.3 (0.61, 2.6)
Any STH					
control	0.72 (34/47)	0.79 (30/38)			
intervention	0.61 (27/44)	0.46 (18/39)		0.70 (0.44, 1.1)	0.71 (0.44, 1.1)
Any pathogenic bacteria, virus, protozoa, or STH					
control	0.91 (43/47)	0.95 (36/38)			
intervention	0.93 (41/44)	0.84 (33/38)			
Number					
	Baseline number	24-month number		Unadjusted BL-24M DID estimate	Adjusted BL-24M DID estimate
Pathogenic bacteria (out of 10)					
control	1.5	1.9			
intervention	1.6	1.1		**0.56 (0.32, 0.96)**	**0.55 (0.31, 0.94)**
Pathogenic viruses (out of 5)					
control	0.70	0.63			
intervention	0.52	0.51		1.1 (0.49, 2.4)	1.1 (0.49, 2.4)
Pathogenic protozoa (out of 3)					
control	0.51	0.39			
intervention	0.45	0.36		1.0 (0.40, 2.6)	1.1 (0.50, 2.4)
STHs (out of 2)					
control	1.1	1.0			
intervention	0.80	0.62		0.82 (0.42, 1.6)	0.83 (0.48, 1.4)

Table C5 continued

Sum of pathogenic bacteria, viruses, protozoa, and STHs					
control	3.8	3.9			
intervention	3.3	2.6			

Note: Bold indicates p<0.05. BL: baseline. 24M: 24-month. DID: difference-in-difference. STH: soil-transmitted helminth.

Table C6. DID analysis on individual pathogens excluding crossover compounds

Pathogenic bacteria					
	Baseline Prevalence	24-month Prevalence		Unadjusted BL-24M DID estimate	Adjusted BL-24M DID estimate
EAEC (*aaiC*/*aatA*)					
control	0.43 (20/47)	0.53 (20/38)			
intervention	0.5 (22/44)	0.38 (15/39)		0.62 (0.32, 1.2)	0.54 (0.29, 1.0)
Shigella/EIES (*ipaH*)					
control	0.34 (16/47)	0.34 (13/38)			
intervention	0.16 (7/44)	0.08 (3/39)		0.49 (0.13, 1.9)	0.53 (0.14, 1.9)
ETEC (ST/LT)					
control	0.26 (12/47)	0.34 (13/38)			
intervention	0.36 (16/44)	0.23 (9/39)		0.51 (0.19, 1.3)	0.55 (0.21, 1.4)
EPEC (*bfpA*/*eae*)					
control	0.13 (6/47)	0.24 (9/38)			
intervention	0.23 (10/44)	0.08 (3/39)		**0.19 (0.04, 0.85)**	**0.20 (0.04, 0.87)**
C. difficile (tcdA/tcdB)					
control	0.11 (5/47)	0.18 (7/38)			
intervention	0.18 (8/44)	0.15 (6/39)		0.47 (0.12, 2.0)	0.47 (0.12, 1.9)
Salmonella					
control	0.02 (1/47)	0.11 (4/38)			
intervention	0.05 (2/44)	0.05 (2/39)		0.21 (0.01, 4.2)	0.14 (0.01, 1.7)
STEC (stx1/stx2)					
control	0.04 (2/47)	0.08 (3/38)			
intervention	0 (0/44)	0.03 (1/39)		NA	

Table C7 continued

Campylobacter jejuni/coli					
control	0.15 (7/47)	0.05 (2/38)			
intervention	0.09 (4/44)	0.08 (3/39)		2.1 (0.36, 12)	1.9 (0.27, 14)
Yersinia spp.					
	0.04 (2/47)	0.05 (2/38)			
	0.02 (1/44)	0.05 (2/39)		1.9 (0.09, 41)	2.6 (0.14, 49)
Vibrio Cholerae					
control	0 (0/47)	0 (0/38)			
intervention	0 (0/44)	0 (0/39)		NA	
Pathogenic viruses					
Adenovirus 40/41					
control	0.26 (12/47)	0.32 (12/38)			
intervention	0.11 (5/44)	0.08 (3/39)		0.52 (0.11, 2.5)	0.45 (0.10, 2.0)
Astrovirus					
control	0.26 (12/47)	0.29 (11/38)			
intervention	0.2 (9/44)	0.31 (12/39)		1.4 (0.54, 3.4)	1.4 (0.52, 4.0)
Norovirus (GI/GII)					
control	0.06 (3/47)	0.03 (1/38)			
intervention	0.07 (3/44)	0.03 (1/39)		NA	
Rotavirus A					
control	0.13 (6/47)	0 (0/38)			
intervention	0.14 (6/44)	0.10 (4/39)		NA	
Sapovirus (I/II/IV/V)					
control	0 (0/47)	0 (0/38)			
intervention	0 (0/44)	0 (0/39)		NA	
Pathogenic protozoa					
Giardia duodenalis					
control	0.43 (20/47)	0.34 (13/38)			
intervention	0.39 (17/44)	0.26 (10/39)		0.88 (0.37, 2.1)	0.97 (0.41, 2.3)
Cryptosporidium parvum					
control	0.06 (3/47)	0.05 (2/38)			
intervention	0.07 (3/44)	0.08 (3/39)		1.4 (0.13, 15)	1.2 (0.12, 13)
Entamoeba histolytica					
control	0.02 (1/47)	0 (0/38)			
intervention	0.02 (1/44)	0.03 (1/39)		NA	NA

Table C8 continued

STHs					
Ascaris lumbricoides					
control	0.68 (32/47)	0.79 (30/38)			
intervention	0.59 (26/44)	0.44 (17/39)		0.64 (0.40, 1.0)	0.65 (0.40, 1.1)
Trichuris trichiuria					
control	0.38 (18/47)	0.21 (8/38)			
intervention	0.2 (9/44)	0.18 (7/39)		1.6 (0.50, 5.0)	1.6 (0.51, 4.9)

Note: Bold indicates p<0.05. BL: baseline. 24M: 24-month. DID: difference-in-difference. STH: soil-transmitted helminth.

Table C9. Animal ownership at baseline and 24-month follow-up

	Baseline		24-month	
Animal presence	Control	Intervention	Control	Intervention
Chickens/ducks	11% (5/47)	18% (8/44)	6.8% (3/44)	20% (9/44)
Dogs	8.5% (4/47)	6.8% (3/44)	25% (11/44)	185 (8/44)
Cats	51% (24/47)	52% (23/44)	30% (13/44)	30% (13/44)
Other	2.1% (1/47)	4.5% (2/44)	2.3% (1/44)	0% (0/44)

APPENDIX D. SUPPLEMENTAL MATERIALS TO CHAPTER 7

Table D1. Descriptions of molecular assays

Assay	Probe concentration (nM)	Forward / reverse primer concentration (nm)	Template volume (dilution)	Cycling conditions
MS2 (reverse transcriptase PCR, ABI 7500)	250	900	1 µL (none)	1. 45°C 10 minutes 2. 94°C 10 minutes 3. 40× (94°C 0.5 minutes, 60°C 1 minute) 2°C/s ramp rate; sample volume 40 µL
ybbW (ddPCR, QX 200)	250	900	1 µL (1:100)	1. 95°C 10 minutes 2. 40× (95°C 0.5 minutes, 59°C 2 minutes) 3. 4°C 5 minutes 4. 90°C 5 minutes 5. 4°C hold 1°C/s ramp rate, heated lid 105°C; sample volume 40 µL
ipaH (ddPCR, QX 200)	250	900	4 µL (none)	1. 95°C 10 minutes 2. 40× (95°C 0.5 minutes, 58.7°C 1 minute) 3. 98°C 10 minutes 4. 4°C hold 2°C/s ramp rate, heated lid 105°C; sample volume 40 µL
beta-giardin (ddPCR, QX 200)	250	900	4 µL (none)	nutes, 58.7°C 1 minute) 2°C/s ramp rate, heated lid 105°C; sample volume 40 µL

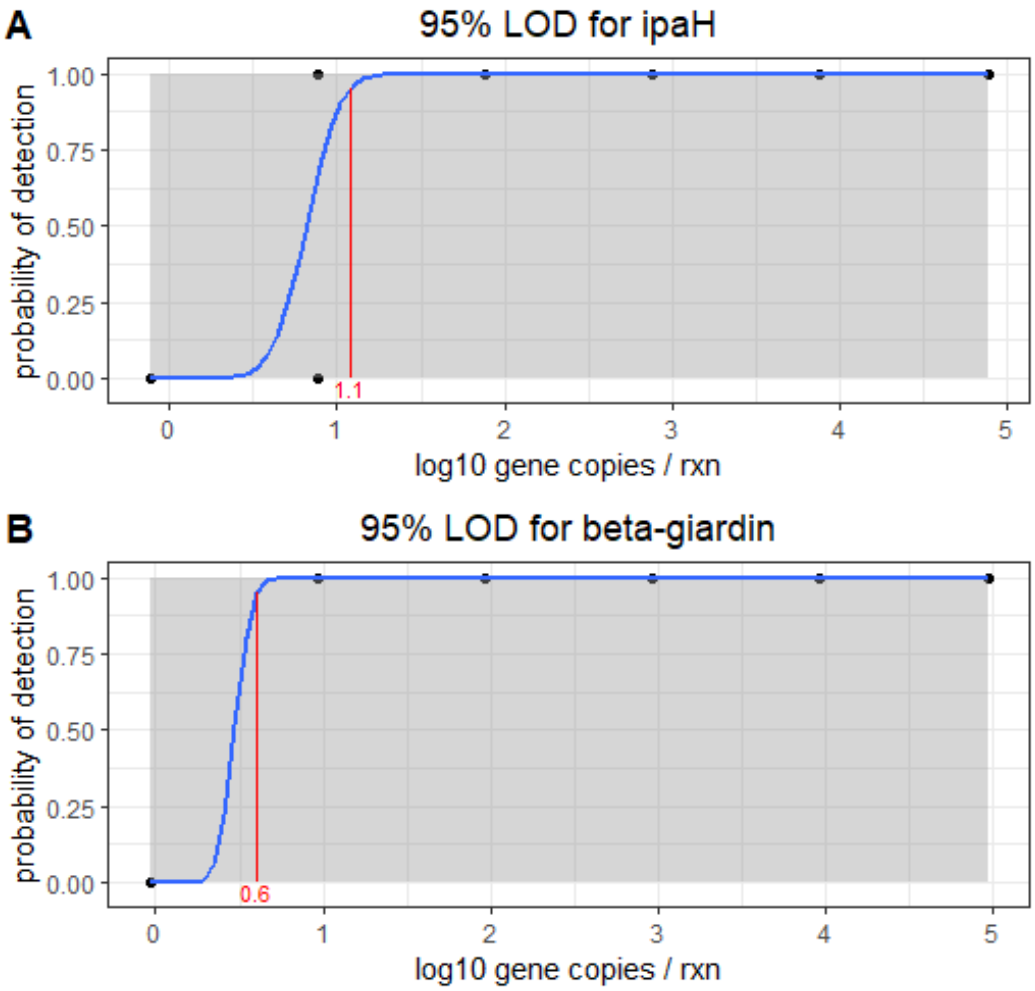

Figure D1. 95% LOD determination for droplet digital PCR assays (A) *ipaH* and (B) *beta-giardin*

D.1 Equations used in QMRA model

Dose response harmonization and viability

Equation 1: For *Giardia duodenalis*

dose$_{Giardia}$ (cysts / day) = *beta-giardin* (gene copies/gram soil) × 1 cyst / 16 gene copies × (Viable$_{E.\ coli}$ / *ybbW*) × soil ingested (grams / day)

Where:

beta-giardin is the density of the *beta-giardin* gene (gene copies / gram soil) stochastic

Viable$_{E.\ coli}$ is the culturable *E. coli* count observed in soils (CFU / gram soil), stochastic

ybbW is the density of the *ybbW* gene in soils (gene copies / gram soil), stochastic

Soil ingested is the amount of soil ingested per day (grams / day), stochastic

Equation 2: For *Shigella spp.*

Dose$_{Shigella}$ (CFUs / day) = *ipaH* (gene copies/gram soil) × *ipaH*$_{\text{gene copies/genome}}$ × (Viable$_{E.\ coli}$ / *ybbW*) × soil ingested (grams / day)

Where:

ipaH is the density of the *ipaH* gene (gene copies / gram soil) stochastic

ipaH$_{\text{gene copies/genome}}$ is the *ipaH* gene copies per *Shigella* genome, stochastic

Viable$_{E.\ coli}$ is the culturable *E. coli* count observed in soils (CFU / gram soil), stochastic

ybbW is the density of the *ybbW* gene in soils (gene copies / gram soil), stochastic

Soil ingested is the amount of soil ingested per day (grams / day), stochastic

Dose response equations

Equation 3: For *Giardia duodenalis*

$$P_{inf} = 1 - \exp(-k * dose)$$

Where:

P_{inf} is the probability of infection

k is a parameter of the exponential model fit to dose response data (stochastic)

dose is the ingested dose of viable cysts (see equation 1), (stochastic)

Equation 4: For *Shigella spp.*

$$P_{inf} = 1 - [1 + dose \times (2^{1/\alpha} - 1)/N_{50}]^{-\alpha}$$

Where:

P_{inf} is the probability of infection

alpha is parameter of the approximate beta Poisson model, stochastic

N_{50} is the median infectious dose, stochastic

dose is the ingested dose of viable *Shigella* (see equation 2), stochastic

Table D2. Estimated daily infection risk

	Model output using soil ingestion estimates from US EPA Exposure Factors Handbook				
	Estimated daily risk of *Giardia duodenalis* infection				
	Percentile	**10th**	**50th**	**90th**	**MapSan 24-month Prevalence**
Age	<6 months	8.4×10^{-6} %	4.3×10^{-4} %	2.0×10^{-2} %	13%
	6-11 months	1.0×10^{-6} %	5.3×10^{-4} %	2.7×10^{-2} %	22%
	12-23 months	1.9×10^{-5} %	9.1×10^{-4} %	4.2×10^{-2} %	59%
	24-71 months	1.0×10^{-5} %	5.1×10^{-4} %	2.8×10^{-2} %	73%
	Geophagy (12-71 months)	1.1×10^{-2} %	5.6 %	25%	70%
	Estimated daily risk of *Shigella* spp. infection				
Age	<6 months	4.0×10^{-6} %	1.9×10^{-4} %	9.1×10^{-3} %	5.0%
	6-11 months	4.9×10^{-6} %	2.4×10^{-4} %	1.3×10^{-2} %	21%
	12-23 months	9.2×10^{-6} %	4.0×10^{-4} %	2.0×10^{-3} %	36%
	24-71 months	4.6×10^{-6} %	2.4×10^{-4} %	1.3×10^{-3} %	68%
	Geophagy (12-71 months)	5.5×10^{-3} %	0.26 %	9.6%	62%
	Model output using soil ingestion estimates from Kwong *et al*.				
	Estimated daily risk of *Giardia duodenalis* infection				
	Percentile	**10th**	**50th**	**90th**	**MapSan 24-month Prevalence**
Age	3-5 months	9.4×10^{-5} %	4.6×10^{-3} %	0.21%	13%
	6-11 months	1.3×10^{-4} %	6.4×10^{-3} %	0.31%	22%
	12-23 months	1.4×10^{-4} %	6.7×10^{-3} %	0.31%	59%
	36-47 months	1.0×10^{-4} %	4.9×10^{-3} %	0.23%	75%
	Estimated daily risk of *Shigella* spp. infection				
Age	3-5 months	4.3×10^{-5} %	2.1×10^{-3} %	9.4×10^{-2} %	5.0%
	6-11 months	6.2×10^{-5} %	2.9×10^{-3} %	0.13%	21%
	12-23 months	6.5×10^{-5} %	3.1×10^{-3} %	0.14%	36%
	36-47 months	4.9×10^{-5} %	2.2×10^{-3} %	0.10%	73%

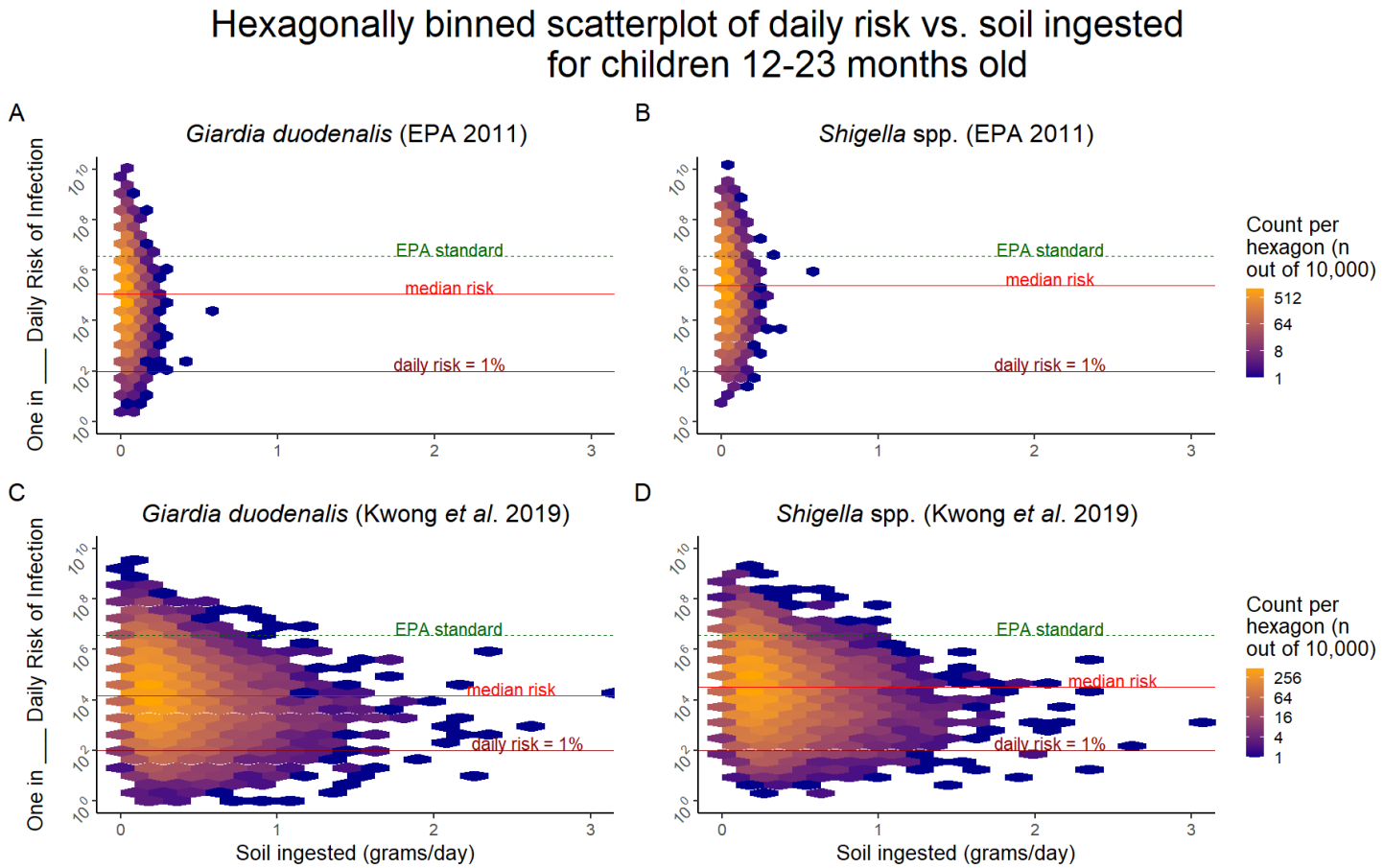

Figure D2. Hexagonally binned scatterplot of daily risk vs soil ingested for children 12-23 months old

Note: EPA standard is the daily risk equivalent of a 1 in 10,000 annual infection risk and a 1% daily risk of infection is equivalent to an annual risk of 97%.

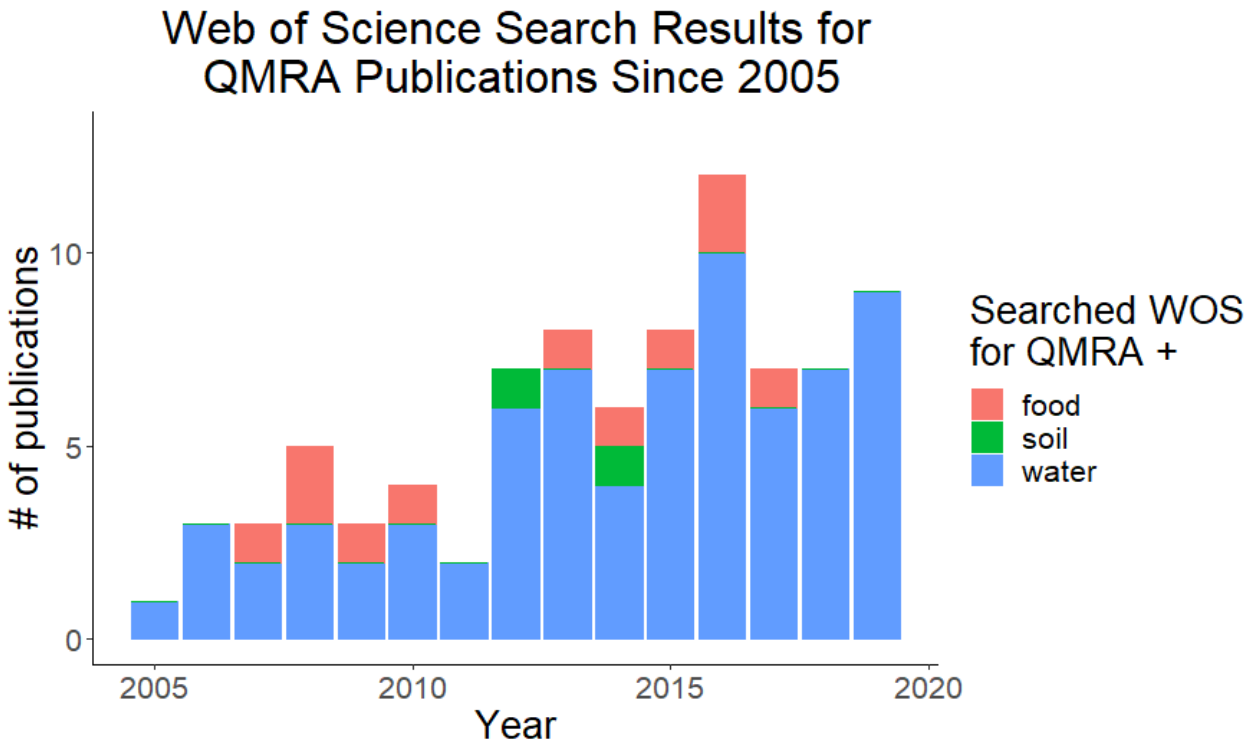

Figure D3. Results from Web of Science search (https://www.webofknowledge.com/) for publications with titles containing the words "QMRA" OR "quantitative microbial risk assessment", and either "water", "soil", or "food"

APPENDIX E. A LOCALIZED SANITATION STATUS INDEX AS A PROXY FOR FECAL CONTAMINATION IN URBAN MAPUTO, MOZAMBIQUE

Citation for the published manuscript:

Capone, D.; Adriano, Z.; Berendes, D.; Cumming, O.; Dreibelbis, R.; Holcomb, D. A.; Knee, J.; Ross, I.; Brown, J. A Localized Sanitation Status Index as a Proxy for Fecal Contamination in Urban Maputo, Mozambique. *PLoS One* **2019**, *14* (10).

E.1 ABSTRACT

Sanitary surveys are used in low- and middle-income countries to assess water, sanitation, and hygiene conditions, but have rarely been compared with direct measures of environmental fecal contamination. We conducted a cross-sectional assessment of sanitary conditions and *E. coli* counts in soils and on surfaces of compounds (household clusters) in low-income neighborhoods of Maputo, Mozambique. We adapted the World Bank's Urban Sanitation Status Index to implement a sanitary survey tool specifically for compounds: a Localized Sanitation Status Index (LSSI) ranging from zero (poor sanitary conditions) to one (better sanitary conditions) calculated from 20 variables that characterized local sanitary conditions. We measured the variation in the LSSI with *E. coli* counts in soil (nine locations/compound) and surface swabs (seven locations/compound) in 80 compounds to assess reliability. Multivariable regression indicated that a ten-percentage point increase in LSSI was associated with 0.05 (95% CI: 0.00, 0.11) $\log_{10}$ fewer *E. coli*/dry gram in courtyard soil. Overall, the LSSI may be associated with fecal contamination in compound soil; however, the differences detected may not be meaningful in terms of public health hazards.

E.2 INTRODUCTION

Disparities in sanitation coverage exist across the globe: in North America and Europe 97% of the population have access to at least basic sanitation compared to 28% in Sub-Saharan Africa [144]. The United Nations Joint Monitoring Programme's (JMP) Sustainable Development Goal (SDG) 6 calls for universal access to safely managed sanitation by 2030, which it defines as "the use of improved facilities and where excreta are safely disposed of *in situ* or treated off-site" [144]. One step below safely managed on the JMP sanitation ladder is the basic sanitation service level, defined as "use of unshared improved facilities."

Safely managed sanitation is one of multiple water, sanitation, and hygiene (WASH) interventions designed to serve as a primary barrier to environmental enteric pathogen transmission and, subsequently, reduce enteric infections [23]. Human excreta is more likely to spread infection via multiple interacting pathways when safely managed sanitation is absent [146]. There is increasing interest in soil as an important environmental transmission pathway for enteric pathogens, especially among children who may mouth contaminated hands or objects, or directly ingest soil [5,98,103,200,267]. The pathways through which human excreta spreads to the environment further suggests that soil serves as a sink for enteric pathogens [146,157]. As such, the levels of fecal contamination in soils—and on other household surfaces frequently contacted by children—may provide a useful metric for assessing the fecal waste-related hazards present generally at local household and near-household scales.

Recent large health impact trials found mixed effects of WASH interventions on children's growth and diarrhea [38,39,268]. Fecal-oral pathogens are transmitted through multiple pathways[23,262,269] and recent large health impact trials may have insufficiently reduced the

dose of pathogens ingested by children or failed to reduce a sufficient number of transmission pathways to observe a health impact. Given that children's growth and diarrhea prevalence are distal effects of sanitation, presumably mediated by reductions in fecal contamination, understanding and reducing fecal contamination in soil [100,199,217]—and other environmental matrices [270,271]—may be useful before further expensive health impact trials are conducted. Without changes to other indicators of sanitary quality (e.g. drainage, solid waste management, fecal sludge management, presence of animals, latrine flooding), simple WASH improvements (e.g. providing latrines with only a slab) may be insufficient to reduce exposure risks to fecal-oral pathogens. Reducing environmental fecal contamination may require systems-based approaches [272], including holistic, transformative interventions that ensure effective sequestration of human and animal fecal wastes both at the household and downstream in the sanitation chain.

Sanitary surveys are a systems-based approach to assess the disposal chain of human excreta and sanitary conditions [30,31,273,274]. Many existing sanitary survey instruments are intended to support the development of sanitation master plans or to identify areas in need of sanitation interventions, particularly at neighborhood or city-wide levels [30,31,87,273,274]. A localized (i.e., near-household) sanitary survey may be useful as a proxy for environmental fecal contamination. However, there is limited evidence of the validity of localized sanitary survey instruments as useful and reliable indicators of compound environmental fecal contamination [275].

A major challenge in evaluating environmental fecal contamination with such sanitary survey metrics is the choice of indicator organism or pathogen for reasons of cost and capacity. Statistically representative, quantitative measures of enteric pathogens or

pathogen/fecal indicators in all environmental media of interest in a given setting are both time-consuming and generally prohibitively expensive [276]. Proxy measures of fecal contamination are often useful in approximating sanitary risks and evaluating sanitation status [276]. By comparing sanitary survey scores to the occurrence of *E. coli*, a widely used fecal indicator, in soils and on surfaces, we can evaluate the suitability of such an approach for approximating localized fecal contamination.

The objectives of our study were to (1) design and implement a sanitary survey that systematically quantified the sanitary conditions at compounds enrolled in a sanitation trial in low-income urban communities of Maputo, Mozambique; (2) evaluate whether and how the sanitary survey were associated with localized fecal hazards, as indicated by *E. coli* occurrence in soil and on surfaces from study compounds; and (3) identify other key variables associated with *E. coli* counts in courtyard soils and on surfaces in this setting. Results of this study could inform future sanitary survey validation in other settings.

E.3 MATERIALS AND METHODS

E.3.1 The Maputo Sanitation (MapSan) Trial

The Maputo metropolitan area contains 2.7 million people [149], of which about only 136,000 (5%) are served by a sewer system that is insufficiently funded for adequate maintenance [88]. Among those without sewerage, about 36% use pit latrines and 64% use pour-flush toilets leading to a pit or septic tank [87]. About 14% of on-site sanitation facilities in Maputo are shared by two or more households [74].

The MapSan Trial was a controlled, before-and-after trial to estimate the health impacts of an urban sanitation intervention [90]. The intervention consisted of private pour-flush latrines (to septic tank) shared by multiple households in compounds (Figure E1, Figure A3), which were installed from 2015-2017. Areas of Maputo with a high-water table were excluded from receiving the intervention. Controls used existing shared private latrines throughout the trial. The study area was in densely populated, low-income, unplanned neighborhoods of urban Maputo, Mozambique. The study area is characterized by poor sanitary and environmental conditions, which contribute to a high burden of enteric disease and child mortality [9,10,78,277]. As a purposive, nested sub-study, this study included a selection of both intervention and control compounds enrolled in the MapSan trial.

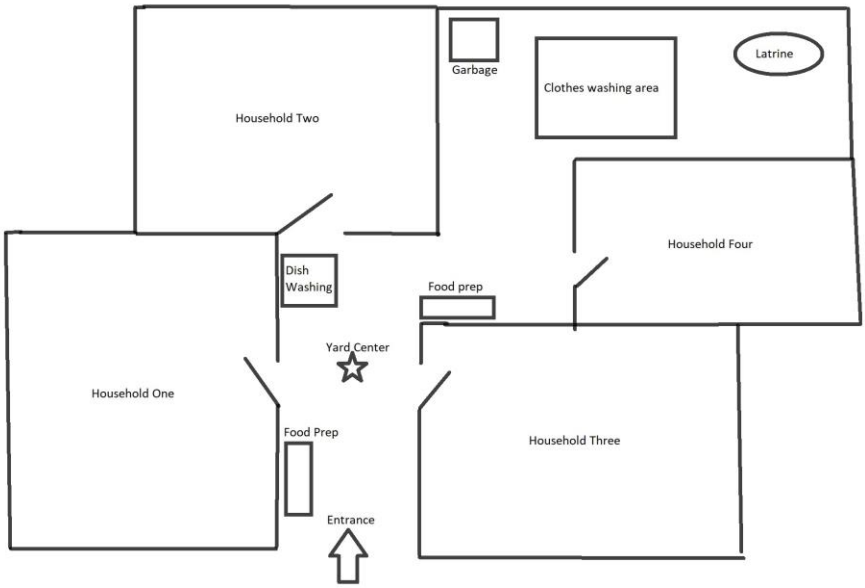

Figure E1. MapSan compound diagram and examples of intra-compound locations

E.3.2 The Localized Sanitation Status Index

We conducted a literature review to identify methodologies to consider for adaptation that yielded six recent sanitary surveys [30,31,87,273,274,278]. These surveys relied on similar inputs: socioeconomic variables [273], habitation characteristics [273,274], water access and availability [30,31,87,273,274], the full disposal chain of human excreta [30,31,87,273,274], solid waste disposal methods [30,31,87,273,274], drainage and waste water conditions [30,87,273,274], latrine sharing [30,87,273], latrine hygienic conditions [30,87,273], the safety of the latrine superstructure [87], and open defecation practices[278]. Of these surveys, we chose to adapt the World Bank's Urban Sanitation Status Index (USSI) because 1) its methodology was the most feasible, and 2) it was locally relevant, as it was developed in Maputo [33].

The USSI was constructed using the guidelines proposed by the Organization for Economic Cooperation for the construction of composite indicators [279]. The USSI was developed based on the theoretical framework proposed by the World Bank's Water and Sanitation Program (WSP), which accounts for the three main steps in on-site sanitation management: containment, emptying and transport, and treatment and disposal [280,281]. WSP recognized sanitation as a series of interlinked services and therefore included "complementary services" as the fourth component to evaluate sanitation status.[87] For each of the four components, WSP conducted a literature review to select the USSI's indicators using the following criteria: (1) appropriate to the study context; (2) data could be easily collected; (3) sensitive to spatial or temporal change; (4) easy to interpret; (5) policy-relevant or actionable [87].

The USSI uses surveys of households and local sanitation experts to calculate nine indicators of sanitary conditions and the overall sanitary score [33,87]. In constructing the Localized Sanitation Status Index (LSSI), we retained 18 of the 20 variables from the USSI.

We did not add any additional variables but did split the *transport safety* (to separate the household and community inputs present in the USSI variable) and *onsite sanitation superstructure* (the USSI used *roof* and *walls* as unique sub-variables in the on-site sanitation superstructure variable, we reported them as two variables for transparency) variables into two variables for each, for a total of 20 unique variables. We excluded the *level of treatment of excreta* variable used in the USSI from the LSSI to avoid including homogenous inputs (there was only one poorly maintained treatment plant in Maputo at the time of survey). Similarly, we excluded the *drainage canals* variable because minimal drainage infrastructure served the study area at the time of survey.

Our adaptation followed the same framework as the USSI, except the outcome of USSI was an average community level sanitation score while we chose to analyze and retain individual household data to produce a localized sanitation score. We designed household and community block leader survey questions to correspond to the 20 input variables of sanitary conditions for the LSSI (Table E1).

We assigned ordinal values ranging from 0 to 1 (in order of poorest to best sanitary conditions) to each survey response for each of the 20 input variables. Intermediate values were split evenly across the range (e.g. ordinal responses of A, B, C, and D were assigned 0, 0.33, 0.67, and 1, respectively). We weighted the 20 input variables according to the previously-implemented USSI in Maputo [33] and used weighted values created by the World Bank for Maputo to calculate the nine indicators of local sanitary conditions (Table E1). The weights for Maputo were created using the Analytic Hierarchy Process technique [282] to estimate the relative importance of each indicator from a questionnaire of 20 local sanitation experts [33,87]. Local sanitation experts included utility and local government

sanitation managers, environmental health officers, NGOs and aid workers, researchers, and provincial/national government personnel. We aggregated the nine indicators according to their weight to calculate the LSSI for each compound (Table E1). We provide further detail on variable and indicator aggregation in the supporting information (see appendix of published manuscript).

Recognizing that the development of the within-variable categorial weights, variable weights and indicator weights may have been subjective, we developed a simplified LSSI alternative, the Unweighted LSSI, to compare against the LSSI. We calculated the Unweighted LSSI by a simple average of the 20 LSSI variables.

Table E1. LSSI variables

Component	Indicator	Indicator Weight	Variable	Data Source	Variable Weight	References
Containment	Access to Infrastructure	14.9%	Type of on-site sanitation system	Household survey	0.7	144,146,192,283
			On-site sanitation sharing	Household survey	0.3	91,284
	Containment Safety	8.6%	Structural stability of the facility	Household survey	0.25	283,285
			Type of lining	Household survey	0.25	146,285,286
			On-site sanitation system roof	Household survey	0.125	146,285,287
			On-site sanitation system walls	Household survey	0.125	146,285,287
			Containment effectiveness	Household survey	0.25	146,157,274
			Groundwater level	Community block leader survey	*	146,157,285,286
	Hygiene	12.9%	Hygienic condition of the on-site sanitation system	Household survey	0.4	30,192,273
			Soap and water nearby for handwashing	Household survey	0.3	146,284,288
			Type of lid covering the drop hole	Household survey	0.3	146,157,287
Emptying and Transport	Access to emptying services	18.0%	Intended type of equipment to empty the latrine or septic tank	Household survey	1	1,22,87,157
	Transport safety	7.9%	Local amount of fecal waste transported to WWTP	Household survey	0.5	1,22,87,157
			Neighborhood amount of fecal waste transported to WWTP	Community block leader survey	0.5	1,22,87,157
Final Disposal	Final disposal	14.4%	Quality of disposal management	Household survey	1	22,146,157
			Groundwater level	Community block leader survey	*	146,157,285,286
Complementary services	Access to water supply	7.7%	Water availability for flushing and cleaning	Household survey	1	30,146,289
	Solid Waste Management	7.0%	Local accumulation of solid waste	Household survey	0.8	87,273,285
			Neighborhood accumulation of solid waste	Community block leader survey	0.2	87,273,285
	Storm- and greywater management	8.4%	Local accumulation of storm water	Household survey	0.5	87,285,290
			In-house greywater management	Household survey	0.5	87,290,291

*Groundwater level had no weight. It was used as a multiplier.

E.3.4 Survey groups

This survey took place from December 2017 to July 2018. We trained enumerators to conduct interviews with household residents through a two-day facilitated workshop and during one week of survey piloting in December 2017, and an additional two days of survey piloting in April 2018. We trained enumerators to conduct interviews with community block leaders through a one-day facilitated workshop and one day of survey piloting in May 2018. Enumerators conducted interviews with household residents from April – July 2018 and with community block leaders in June 2018.

All questionnaires were communicated by the enumerators in either Portuguese or the local language, Changana, as requested by the respondent. Our sampling frame included one household respondent from each compound enrolled in the MapSan trial that had completed the 12-month follow-up household survey.[90] We recognized that MapSan respondents were a relatively homogenous group (women with young children). Therefore, we aimed to survey a second non-MapSan household respondent from each compound, who we identified as an adult resident of the third household on the right of the compound entrance.

In ArcGIS (ESRI, Redlands, CA) we laid a grid of 40 points across the MapSan trial area approximately 300 meters apart and determined the community block each point was located in. Enumerators visited the corresponding community block leaders and surveyed them at their homes. Community block leaders are volunteers who serve as the lowest level government officials in Maputo, and their responsibilities include mobilizing residents to look after public infrastructure and cleanliness [87]. We matched household survey responses

to the nearest community block leader by GPS location for neighborhood-level LSSI inputs (Figure E2).

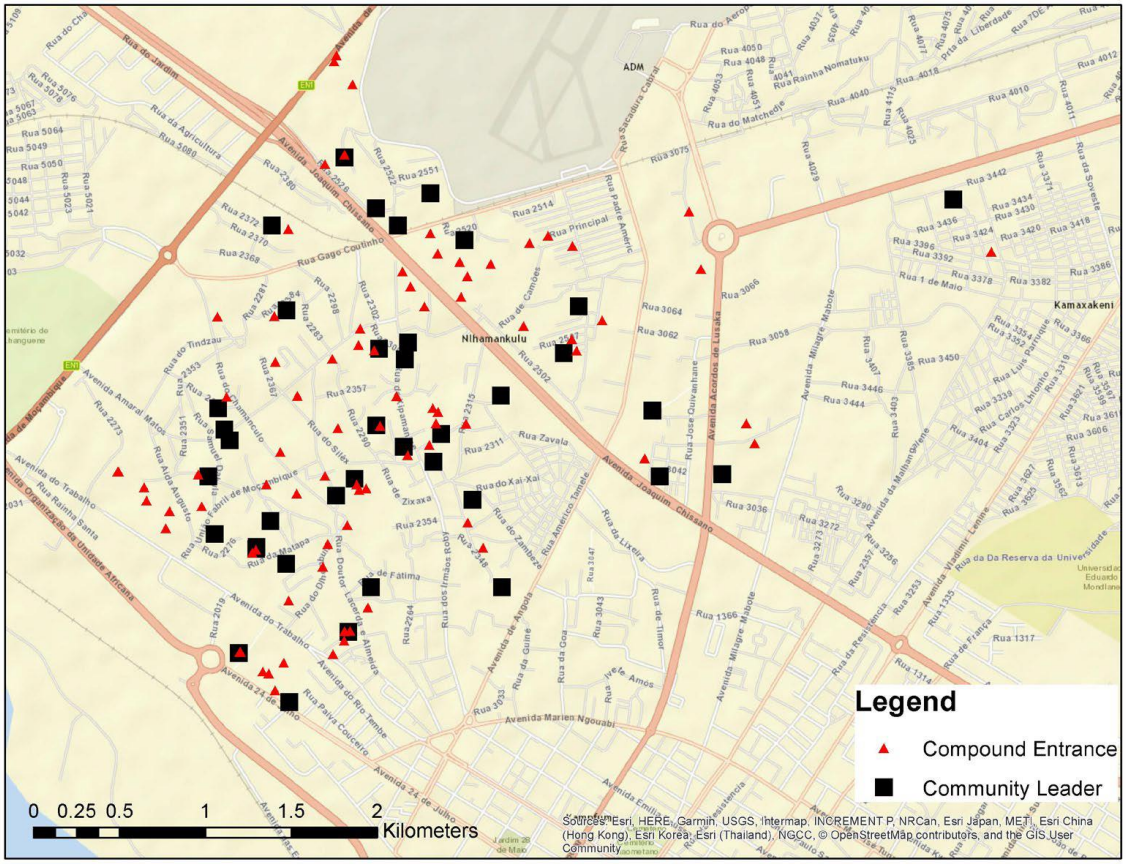

Figure E2. Map of study area

E.3.5 Environmental sampling site selection

We calculated preliminary LSSI scores to identify compounds for environmental sampling by applying the LSSI methodology to household survey data collected during the most recent (24-month) follow-up visits of the MapSan trial. In calculating the preliminary LSSI, we ignored neighborhood and certain household-level variables that were not collected as part of the MapSan survey conducted from 2017-2018. Based on resource constraints we

aimed *a priori* to sample from 80 total compounds: those with the 40 highest and 40 lowest scores on the preliminary LSSI to test the hypothesis that the LSSI varies with objective measures of fecal contamination. The selection of compounds at the extremes of LSSI equipped the study with the greatest power to detect differences in environmental fecal contamination between relatively low and high LSSI scores. We conducted environmental sampling of soils and surfaces from May – June 2018.

E.3.6 Soil sampling

At each compound we collected nine soil samples at the following locations, as identified by an adult member of a household enrolled in the MapSan trial: 1) the most frequently used compound entrance; 2) the household entrance, 3) the latrine entrance; 4) the food preparation area; 5) the dish-washing area; 6) clothes washing area; and 7) the area solid waste was stored; 8) the center of the compound yard we estimated by approximating the midpoint of all the household entrances in a compound; and 9) a second household entrance, from a household not enrolled in the MapSan study, selected by locating a household entrance across the compound yard from the first household entrance. If there was no household across the compound yard from the first household (sample location 9), we selected the household entrance that was farthest away from the first household entrance. We collected all soil samples using a metal scoop that was disinfected with 10% bleach and 70% ethanol between uses. For each sample, we used the metal scoop to homogenize a 10 cm x 10 cm x 1 cm volume of soil, which we transferred into one 5-mL cryotube and three 2-mL cryotubes. Soil samples remained on ice packs after collection and were processed within 6 hours of collection. A soil sample was recorded as "moist" or "dry" based on whether it was visibly wet at the time of collection. Using an estimate of

the sun's trajectory from approximately 9:00 am to 3:00pm on the day sampling took place (sampling took place during these hours each day) and the presence of nearby coverings (e.g. trees and houses), we estimated daily sun exposure, classifying each sample as "shaded", "partially shaded" or in "direct sunlight" (S3 Text).

Bacteria were eluted from soil using modified methods from Boehm *et al.* [292], similar to methods reported elsewhere [96,97,293]. Briefly, we eluted approximately one gram of soil in 100 mL of distilled water using a 532-mL self-standing Whirl-Pak bag (Nasco, Fort Atkinson, WI). We manually shook soil samples for two minutes and then allowed samples to settle for 15 minutes. We aliquoted one mL of supernatant onto Compact Dry plates for quantification of *E. coli* (Compact Dry^{TM} EC, VWR, Vienna, Austria). We incubated the Compact Dry plates at 37^{o}C for 24 hours as per the manufacturer's instructions. We processed a separate one-gram soil sample from the same cryotube for replicate analysis of each sample and ran a negative control for every 9 soil samples. When one or both replicate samples yielded colonies too numerous to count, we tested a third sample from the same cryotube using a 1:15 dilution of the supernatant. We measured moisture content of soil samples using the microwave oven method [96,98,294]. We calculated *E. coli* counts in colony forming units (CFUs) per gram dry soil by a simple average of the two replicate values. Based off the manufacturer's instructions and the dilutions used, the lower limit of detection was 2 $\log_{10}$ CFU *E. coli* per gram of soil, not accounting for moisture content, and the upper limit of detection was 6.48 $\log_{10}$ CFU *E. coli* per gram of soil.

E.3.6 Swab sampling

At each compound we collected fourteen swab samples at seven locations that were identified by an adult in a household enrolled in the MapSan trial. The household member indicated or provided: 1) the most frequently used compound entrance door or door frame, 2) the household entrance door, 3) latrine entrance door or door frame, 4) a food preparation surface, 5) a plate used to serve food, 6) a plastic chair (we swabbed the horizontal seat surface), and 7) the most frequent play toy of a child from the subject's household. We recorded whether each surface was visibly dirty at the time of sampling. We swabbed adjacent surface areas of 100 cm^2 and $10cm^2$ using a method adapted from Hedin *et al*. and similar to other studies [98,295]. We swabbed each surface with two sterile nylon flocked swabs (Copan Diagnostics, Murrieta, CA). First, we wetted a swab with sterile ¼ strength Ringer's solution (MilliporeSigma, Burlington, MA) and swabbed the entire surface in the horizontal, vertical and diagonal directions. Then we repeated this process on the same surface using a dry swab. We cut the swab end of the wet and dry swabs using scissors sterilized with 10% bleach and 70% ethanol and inserted the swabs into an Ojal Test Kit (Ojal Water Technologies Pvt. Ltd, Bangalore, India, www.ojalwatertest.com), an *E. coli* test that uses Aquatest medium [296,297] to produces a color change in the presence of *E. coli* (S4 Text). We added either 100 mL or 10 mL of distilled water to the Ojal test kits with the swabs in them, according to the manufacturer's instructions, and then shook samples for two minutes to elute *E. coli* from the swabs. The limit of detection from this test was ≥1 *E. coli* per 10 cm^2 and ≥1 *E. coli* per $100cm^2$. We ran a blank control of only distilled water and a second control containing distilled water and a swab wetted in ¼ strength Ringer's solution for every seven samples processed. We incubated the Ojal Test kits at 37^oC for 24 hours, per the manufacturer's instructions, before reading.

E.3.7 Data analysis

We analyzed data in R version 3.5.0 (R Foundation for Statistical Computing, Vienna, Austria). To account for nested clusters of households within clusters of compounds we used linear mixed-effect models (LMM) on $\log_{10}$-transformed values of CFU *E. coli* per dry gram of soil to perform linear regression modelling, and generalized linear mixed-effect models (GLMM) on binary detect/non-detect *E. coli* in soil, and binary detect/non-detect *E. coli* on surfaces to perform Poisson regression modelling. In our models, *E. coli* concentration or detect/non-detect was our dependent variable and the LSSI was our independent variable. We used the "lme4" package in R for regression analysis and used a Poisson (log) distribution for calculation of unadjusted risk ratios (RR) and adjusted risk ratios (aRR) [298].

We *a priori* decided to adjust for sunlight, location of the soil sample in the courtyard, a compound's wealth index, and presence of chickens and ducks[299], as literature suggests these variables may be important confounders [97,98,211]. We did not adjust for soil moisture as both sunlight and the location of a soil sample in the courtyard were associated with soil moisture and moisture was already accounted for by normalizing *E. coli* concentrations by moisture content (per gram dry soil). *A priori* we decided to evaluate associations between *E. coli* in soil and the LSSI score continuously and by quartiles. Given the low levels of *E. coli* detected on surfaces and suggested confounders from a previous study [98], we decided to analyze the detection/non-detection of *E. coli* on surfaces and adjusted for visible dirt on the surface, intra-compound location, and wealth [98].

We assigned *E. coli* concentrations in non-detect soil samples to half the value of the LLOD [51,217] and we did not observe any samples with *E. coli* concentrations above the upper limit of detection. We calculated household wealth using eight of the ten inputs from the Simple Poverty Scorecard for Mozambique [166]. We excluded number of beds and latrine type from our calculation of household wealth because of limited data and latrine type due to our cross sectional design [167]. When we surveyed two households in a compound, we used the mean wealth score as the compound wealth score and the mean LSSI as the compound LSSI.

E.3.8 Ethical approvals

Before conducting a survey with an adult household member or *a* community block leader we obtained written informed consent from the respondent. We obtained verbal consent from the head of a compound to perform environmental sampling and requested permission to sample from all compound heads at least one day in advance. The study protocols were approved by the Comité Nacional de Bioética para a Saúde (CNBS), Ministério da Saúde (333/CNBS/14, 81/CNBS/18), the Ethics Committee of the London School of Hygiene and Tropical Medicine (Reference # 8345) and the Institutional Review Board of the Georgia Institute of Technology (Protocol # H15160, # H18027). The associated MapSan trial has been registered at ClinicalTrials.gov (NCT02362932).

E.4 RESULTS

E.4.1 Household characteristics

We visited 147 households at 80 MapSan compounds (13 compounds lacked a second household to interview) and conducted interviews with 133 households at 75 MapSan compounds (three respondents did not consent and 11 had moved away). The median amount of time respondents lived in their home was nine years and the average was 14 years. Compounds contained an average of four families, 17 people, two children under the age of five, and scored 33 out of 81 (Standard Deviation (SD) = 11) on the Mozambique Simple Poverty Scorecard [166]. We observed human feces in the compound yard or on the floor of the on-site sanitation system at 11% (n=9) of compounds, used children's diapers on the ground or in a pile of garbage at 13% of compounds (n=10), and standing water at 49% (39) compounds. We observed animals in 59% (n=47) of compounds consisting of cats (n=32, [40%]), chickens (n=12, [15%]), ducks (n=8, [10%]), dogs (n=7, [9%]), and pigeons (n=1, [1%]). The on-site sanitation systems at the 80 environmental sampling compounds were predominantly pour-flush to pit or septic tank (n=50, [63%]), while 16% (n=13) possessed pit latrine with concrete slab, and 21% (n=17) possessed a pit latrine without a concrete slab. Additionally, 39 of 40 community block leaders (98%) consented to an interview.

E.4.2 Soils

We collected 720 soil samples from 80 MapSan compounds and detected *E. coli* in 74% of samples with a mean concentration of 4.10 $\log_{10}$ CFU *E. coli* per gram of dry soil (standard deviation = 4.78 $\log_{10}$) and a median of 2.77 $\log_{10}$ CFU *E. coli* per gram of dry soil (range = no detect (ND), 6.14 $\log_{10}$). The mean difference between the replicate soil samples analyzed from each location was 3.76 $\log_{10}$ CFU *E. coli* per gram of dry soil, the median was 2.50 $\log_{10}$ CFU *E. coli*, and the Pearson's correlation coefficient was 0.84. We

most frequently detected *E. coli* in soils from washing areas for clothes (91%) and dishes (90%), while least frequently detected *E. coli* in soils at the compound center (60%) and the non-MapSan household entrance (59%) (Table E2). Among intra-compound locations, the highest average *E. coli* concentration was found at the dishwashing area (mean 4.54 $\log_{10}$ CFU *E. coli*), while the center of the compound yard had the lowest concentrations (mean 3.66 $\log_{10}$ CFU *E. coli*). We noted 65% of samples as visibly wet at the time of sampling and 35% as visibly dry; we most frequently observed soil from the clothes washing area (85%, [n=68/80]) and dishwashing area (90%, [n=72/80]) as visibly wet. We recorded that 13% (95) of sample locations experienced complete sunlight throughout the day, 30% (288) both direct sunlight and shade, and 47% (337) remained completely shaded. We estimated sun exposure status to be similar across intra-compound locations, except for the center of the compound yard which was estimated to be in full sun (29%, [n=23/80) more often than other locations and the food preparation area which was estimated to be complete shade (65%, [n=52/80]) more often than the other locations.

Table E2. CFU *E. coli* counts at intra-compound locations

Intra-compound location	≥LLOD	$\geq 10^3$	$\geq 10^4$	Mean ($\log_{10}$)	SD	Median ($\log_{10}$)	Range
Clothes Washing Area	91%	60%	20%	4.08	4.49	3.28	(ND, 5.30)
Dish Washing Area	90%	60%	26%	4.54	5.20	3.21	(ND, 6.14)
Garbage Storage Area	81%	54%	26%	4.35	4.71	3.06	(ND, 5.48)
Latrine Entrance	76%	51%	18%	3.96	4.40	3.05	(ND, 5.29)
MapSan Household Entrance	73%	36%	8%	3.74	4.35	2.42	(ND, 5.24)
Compound Entrance	69%	40%	16%	3.98	4.55	2.48	(ND, 5.46)
Food Prep Area	69%	31%	13%	3.98	4.48	2.42	(ND, 5.25)
Compound Center	60%	24%	8%	3.66	4.26	1.89	(ND, 5.14)
Non-MapSan Household Entrance	59%	33%	11%	3.73	4.18	2.32	(ND, 4.97)
All Locations	74%	43%	16%	4.10	4.78	2.77	(ND, 6.14)

E.4.3 Swabs

We swabbed adjacent 100cm^2 and 10cm^2 surfaces at 560 locations in 80 MapSan compounds, of which 23% appeared visibly dirty. The Ojal Test yielded *E. coli* concentrations of ≥1 *E. coli* per 100cm^2 at 3.4% of the 100cm^2 surfaces and ≥1 *E. coli* per 10cm^2 at 2.9% of the 10cm^2 surfaces. We detected *E. coli* from either the 100cm^2 or 10cm^2 surface at 5.4% of swab locations.

E.4.4 The Complete LSSI

LSSI scores ranged from 0.20 to 0.91, with a mean of 0.55 (SD = 0.20) and the distribution was bimodal (Figure E3). In the 50 compounds where we interviewed two respondents, the average intra-compound LSSI variation between respondents was 0.12 (median: 0.06).

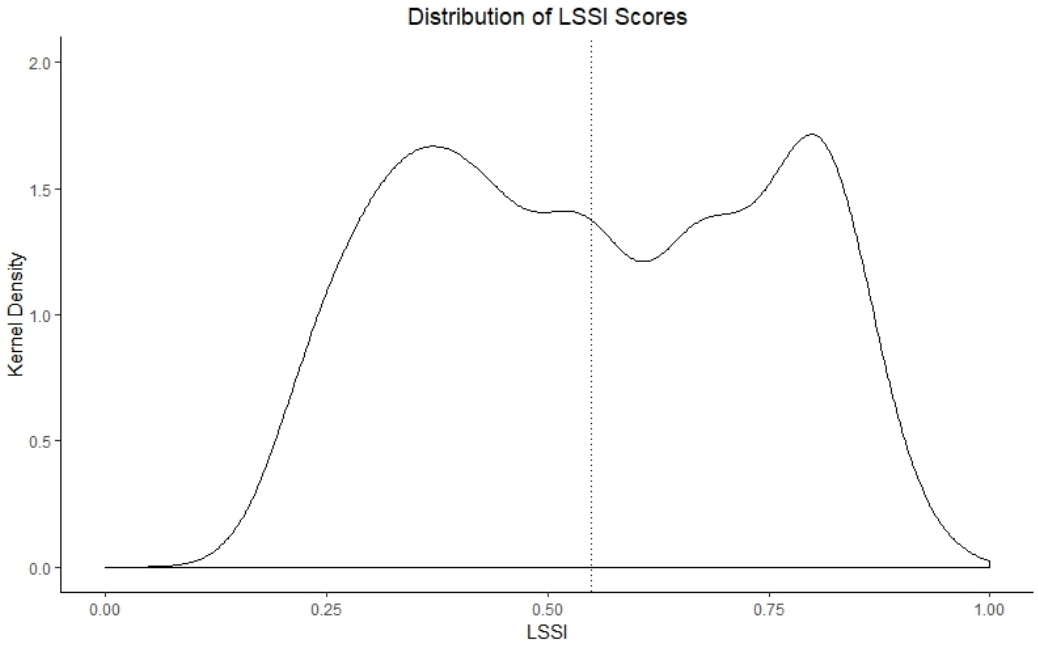

Figure E3. Kernel density plot of complete LSSI results

E.4.5 Continuous E. coli Counts

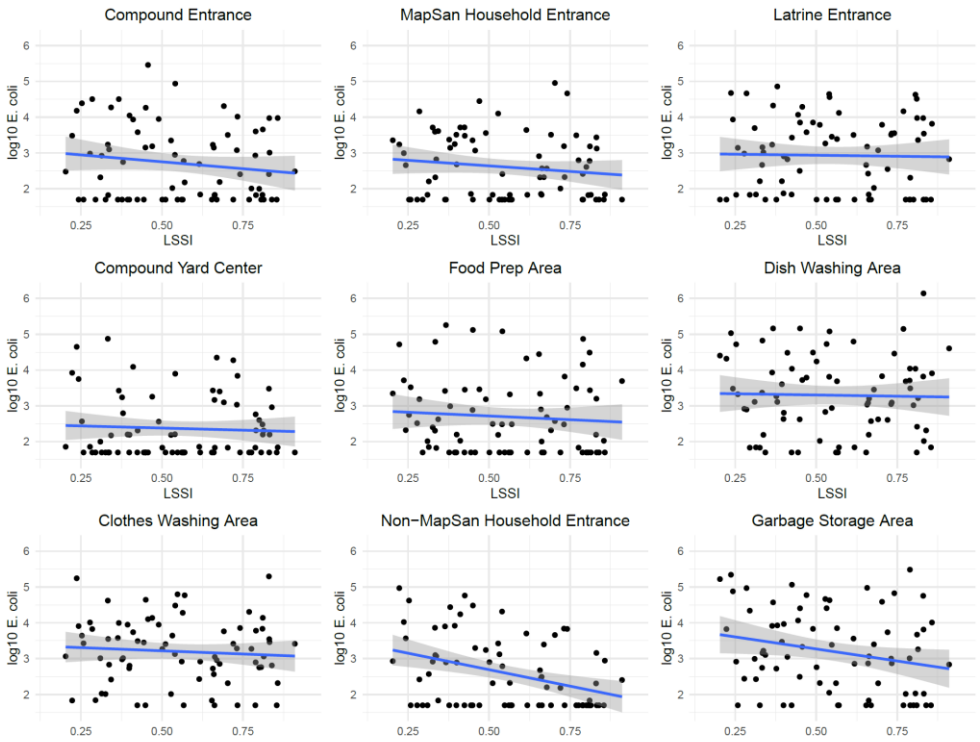

Figure E4. Comparison of the LSSI with *E. coli* counts at the 9 intra-compound locations

Using multivariable regression and adjusted for sun exposure status, intra-compound location, presence of chickens and ducks, and household wealth, a ten-percentage point increase in the LSSI was associated with 0.05 $\log_{10}$ fewer CFU *E. coli* per gram dry soil (95% CI: -0.10, 0.00; Table E3). However, *E. coli* counts in soil were generally heterogenous across the range of LSSI scores (Figure E4). Similarly, a ten-percentage point increase in the Unweighted LSSI was associated with 0.07 $\log_{10}$ fewer CFU *E. coli* per gram dry soil (95% CI: -0.13, -0.01). Four of the 20 LSSI variables were individually associated with $\log_{10}$-transformed *E. coli* counts in soil. A ten-percentage point increase in the *on-site sanitation sharing* variable was associated with 0.06 $\log_{10}$ fewer CFU *E. coli* per gram dry soil (95% CI: -0.10, -0.02), the *groundwater level* variable was associated with 0.03 fewer $\log_{10}$ fewer CFU *E. coli* per gram dry soil (95% CI: -0.06, 0.00), the *quality*

of disposal management variable was associated with 0.05 $\log_{10}$ fewer CFU *E. coli* per gram dry soil (95% CI: -0.09, -0.01), and the *neighborhood accumulation of solid waste* variable was associated with 0.03 $\log_{10}$ fewer CFU *E. coli* per gram dry soil (95% CI:-0.06, 0.00) (Table E4). We did not observe significant associations between the LSSI, when divided by quartile, and *E. coli* concentrations in soil. Adjusted *E. coli* concentrations in soil were significantly associated with shade (higher in full shade vs. full sun), moisture (higher in visibly wet vs. dry soil), and chicken presence (higher with chickens present).

Table E3. Uni- and multi-variable regression models for $\log_{10}$-transformed *E. coli* concentrations in soil and adjusted for sunlight, intra-compound location, compound wealth, chickens and ducks

Soil Covariates	Description	Reference	Univariable β (95% CI)	Multivariable β (95% CI)
Complete LSSI	Localized Sanitation Status Index	Ten-percentage point increase	-0.06 (-0.13, 0.00)	-0.05 (-0.11, 0.00)
Unweighted LSSI	Simple average of the 20 LSSI variables		-0.09 (-0.17, -0.01)	-0.07 (-0.13, -0.00)
LSSI: Q2	LSSI divided into quartiles	Q1	-0.03 (-0.40, 0.34)	0.01 (-0.30, 0.31)
LSSI: Q3			-0.40 (-0.77, -0.03)	-0.29 (-0.60, 0.02)
LSSI: Q4			-0.31 (-0.68, 0.06)	-0.25 (-0.56, 0.07)
Sunlight: partial sun	Estimated daily sun exposure: full sun, partial sun, full shade	Full sun	0.19 (-0.04, 0.42)	0.13 (-0.10, 0.35)
Sunlight: full shade			0.47 (0.23, 0.71)	0.39 (0.16, 0.62)
Moisture	Soil sample classified as "visibly wet" or "dry"	Dry	0.97 (0.83, 1.11)	0.83 (0.69, 0.98)
Compound entrance	One of nine sample locations where soil was collected from each compound	Center of the compound yard	0.35 (0.08, 0.61)	0.29 (0.02, 0.56)
MapSan household entrance			0.22 (-0.04, 0.49)	0.15 (-0.13, 0.42)
Non-MapSan household entrance			0.21 (-0.05, 0.47)	0.14 (-0.13, 0.41)
Latrine entrance			0.58 (0.31, 0.84)	0.45 (0.18, 0.73)
Food preparation area			0.27 (0.00, 0.53)	0.19 (-0.08, 0.47)
Dish washing area			0.89 (0.63, 1.15)	0.82 (0.55, 1.10)
Clothes washing area			0.86 (0.60, 1.12)	0.75 (0.48, 1.02)
Garbage storage area			0.80 (0.54, 1.06)	0.74 (0.47, 1.01)
Wealth Index	Wealth quartile	1-quartile increase	-0.14 (-0.25 -0.02)	-0.09 (-0.19, 0.01)
Chickens	Chickens present in the compound	No chickens	0.94 (0.61, 1.26)	0.66 (0.33, 0.99)
Ducks	Ducks present in the compound	No ducks	0.73 (0.30, 1.16)	0.42 (-0.06, 0.89)

Table E4. Associations between LSSI variables and *E. coli* counts in domestic soils

		Univariable		Multivariable	
Indicator	**Reference**	**Beta**	**95% CI**	**Beta**	**95% CI**
Access to infrastructure	Ten percentage point increase	**-0.09**	**(-0.17, -0.01)**	**-0.07**	**(-0.13, -0.00)**
Containment safety		-0.05	(-0.11, 0.01)	-0.02	(-0.07, 0.03)
Hygiene		-0.04	(-0.09, 0.02)	-0.03	(-0.07, 0.01)
Access to emptying services		-0.02	(-0.05, 0.01)	-0.01	(-0.04, 0.01)
Transport safety		-0.01	(-0.05, 0.03)	-0.01	(-0.04, 0.03)
Final disposal		**-0.04**	**(-0.08, 0.00)**	**-0.04**	**(-0.08, -0.01)**
Access to water supply		-0.01	(-0.04, 0.02)	-0.01	(-0.04, 0.02)
Solid waste management		**-0.07**	**(-0.13 0.00)**	-0.03	(-0.09, 0.03)
Storm- and greywater management		-0.05	(-0.11, 0.01)	-0.03	(-0.08, 0.01)
Variable	**Reference**	**Beta**	**95% CI**	**Beta**	**95% CI**
Type of on-site sanitation system	Ten percentage point Increase	-0.05	(-0.12, 0.02)	-0.02	(-0.08, 0.03)
Toilet sharing		-0.06	**(-0.11, -0.01)**	**-0.06**	**(-0.10, -0.02)**
Structural stability		-0.02	(-0.06, 0.02)	-0.01	(-0.05, 0.02)
Type of lining		-0.02	(-0.07, 0.02)	-0.01	(-0.05, 0.04)
Superstructure roof		-0.01	(-0.04, 0.02)	-0.01	(-0.03, 0.02)
Superstructure walls		-0.04	(-0.11, 0.03)	-0.03	(-0.09, 0.03)
Containment effectiveness		0	(-0.06, 0.07)	0.03	(-0.02, 0.09)
Groundwater level		-0.03	(-0.07, 0.01)	**-0.03**	**(-0.06, 0.00)**
Hygienic condition		-0.02	(-0.06, 0.01)	-0.02	(-0.05, 0.01)
Soap and water for handwashing		-0.02	(-0.06, 0.02)	-0.01	(-0.05, 0.03)
Type of on-site sanitation lid		-0.01	(-0.05, 0.03)	-0.01	(-0.04, 0.02)
Type of emptying equipment		-0.02	(-0.05, 0.01)	-0.02	(-0.04, 0.01)
Local fecal waste transport		-0.02	(-0.05, 0.02)	-0.01	(-0.04, 0.02)
Neighborhood fecal waste transport		-0.01	(-0.06, 0.05)	0.00	(-0.50, 0.50)
Disposal management		**-0.05**	**(-0.10, 0.00)**	**-0.05**	**(-0.09, -0.01)**
Access to water		-0.01	(-0.04, 0.02)	-0.01	(-0.04, 0.02)
Local solid waste		-0.03	(-0.09, 0.02)	-0.01	(-0.06, 0.04)

Table E5 continued.

Neighbor solid waste		-0.04	(-0.08, 0.01)	**-0.03**	**(-0.06, 0.00)**
Greywater management		-0.02	(-0.07, 0.03)	-0.02	(-0.06, 0.02)
Neighbor stormwater accumulation		-0.03	(-0.7, 0.01)	-0.02	(-0.05, 0.02)

E.4.6 Any E. coli Detection

Using multivariable Poisson regression and adjusted for sun exposure status, intra-compound location, presence of chickens and ducks and household wealth, we found a ten-percentage point increase in the LSSI had no apparent association with detection of *E. coli* (aRR: 0.98, 95% CI: 0.94, 1.02;

Table E6). We did not find any apparent associations between the LSSI divided into quartiles and *E. coli* in soil. Additionally, visibly wet soil was associated with greater risk of detection of *E. coli* in soil.

No covariates were significantly associated with the detection of *E. coli* on compound surfaces in univariable or multivariable regression after controlling for visible dirt on a surface, intra-compound location, and wealth index.

Table E6. Logistic regression models using detect/non-detect *E. coli* as the response variable

Soil Covariates	Reference	RR	aRR
Complete LSSI	Ten-percentage point increase	0.97 (0.93, 1.02)	0.98 (0.94, 1.02)
Unweighted LSSI		0.96 (0.91, 1.01)	0.97 (0.92, 1.02)
LSSI Q2	Quartile 1	0.91 (0.72, 1.16)	0.95 (0.73, 1.22)
LSSI Q3		0.87 (0.68, 1.10)	0.90 (0.70, 1.15)
LSSI Q4		0.82 (0.63, 1.05)	0.84 (0.65, 1.09)
Partial sun	Full Sun	1.24 (0.93, 1.67)	1.19 (0.88, 1.63)
Shade		1.30 (0.98, 1.75)	1.27 (0.94, 1.73)
Visibly wet	Visible Dry	1.84 (1.51, 2.26)	1.77 (1.42, 2.23)
Food Prep Area	Compound yard center	1.15 (0.78, 1.69)	1.12 (0.75, 1.69)
Compound Entrance		1.15 (0.78, 1.69)	1.14 (0.77, 1.71)
MapSan Household Entrance		1.21 (0.83, 1.78)	1.19 (0.80, 1.79)
Non-MapSan Household Entrance		0.98 (0.65, 1.47)	0.96 (0.63, 1.46)
Latrine Entrance		1.27 (0.87, 1.86)	1.23 (0.83, 1.83)
Garbage Storage Area		1.35 (0.93, 1.97)	1.32 (0.91, 1.97)
Dish Washing Area		1.50 (1.04, 2.17)	1.47 (1.01, 2.17)
Clothes Washing Area		1.52 (1.06, 2.20)	1.49 (1.04, 2.19)
Chicken Present	No chickens	1.32 (1.06, 1.63)	1.23 (0.96, 1.56)
Duck Present	No ducks	1.23 (0.94, 1.58)	1.07 (0.75, 1.49)
Wealth Index	1-quartile increase	0.93 (0.73, 1.09)	0.94 (0.87, 1.02)

Compound Surface Covariates	Reference	RR	aRR
LSSI	Ten-percentage point increase	0.97 (0.77, 1.24)	0.97 (0.75, 1.23)
Surface visibly dirty	Not visibly dirty	1.25 (0.47, 2.97)	0.91 (0.31, 2.40)
Plastic chair	Compound Entrance	1.80 (0.62, 5.86)	1.80 (0.62, 5.87)
Food prep surface		0.20 (0.01, 1.24)	0.20 (0.01, 1.23)
Dinner Plate		0.20 (0.01, 1.24)	0.20 (0.01, 1.23)
MapSan Household door		0.40 (0.06, 1.86)	0.40 (0.06, 1.85)
Latrine door		0.60 (0.12, 2.44)	0.60 (0.12, 2.45)
Child's toy		0.80 (0.20, 3.02)	0.83 (0.19, 3.35)
Wealth index	1 quartile increase	1.00 (0.64, 1.56)	1.00 (0.64, 1.57)

E.5 Discussion

At compounds in low-income urban Maputo with sanitation shared by multiple households, our adapted sanitary survey methodology, the LSSI, was associated with continuous measures of *E. coli* from compound soils, but not with binary measures of *E. coli* in soils or from compound surfaces. However, we observed a modest 0.05 $\log_{10}$ CFU decrease in *E. coli* in compound soil per ten-percentage point increase in the LSSI, which is smaller than expected, given the range of WASH characteristics across surveyed sites. Thus, a theoretical compound with an LSSI of zero that improved its sanitary conditions to achieve

an LSSI of one would experience an average reduction in *E. coli* concentrations of only 0.50 $\log_{10}$ per gram dry soil in this setting. These findings are consistent with a large, systematic study of environmental contamination in Bangladesh, where seemingly large changes in sanitation—e.g. the presence vs absence of a latrine—were associated with only a 0.56 $\log_{10}$ reduction of *E. coli* in soil [97]. Animals may also be important contributors to environmental fecal contamination in this setting. Though statistically significant, the observed reductions in *E. coli* concentrations are minimal and may not reflect a meaningful difference in environmental contamination, and potential subsequent risks of exposure to feces-associated enteric pathogens. *E. coli* in soils from this environment were widely detected (74% of samples) and in high concentrations (mean: $\log_{10}$ 4.10), so relative differences in *E. coli* may not reflect actual differences of public health relevance.

Our goal was to assess the potential for an association between a policy-relevant metric in use by the World Bank and by cities in Rwanda, Zambia and Mozambique with measures of fecal contamination [33]. Our results suggest that sanitary surveys may serve as useful proxies for localized environmental fecal contamination; the LSSI encompassed relevant sanitary hazards that impacted the spread of human fecal contamination into the environment, thus an association with measures of *E. coli* in soil was anticipated. However, the LSSI should be improved upon to attempt to produce a proxy for fecal contamination that associates with log-level reductions in environmental fecal contamination of public health significance. The association between the access to infrastructure indicator and measures of *E. coli* in soil was greater than association with the complete LSSI. While important for hygiene, the presence of soap and water for handwashing likely had little impact on the spread of fecal contamination into compound soil. Most households in

Maputo reported never having emptied their on-site sanitation system [74]; emptying frequency is dependent on the type of on-site sanitation system and the depth of the water table such that sanitation facilities in Maputo take on average one to five years to fill up [74]. How compounds intended to empty their on-site sanitation system may not be temporally relevant to a cross-sectional sanitary survey. Future iterations of the LSSI may improve their utility by only including variables with a biologically plausible pathway to contribute to localized fecal contamination. In lieu of expert weights which may be subjective, these pathways could be weighted based on the volume, frequency, and likelihood for fecal contaminations to spread into the environment.

As in other low-income settings globally, results from our adjusted estimates indicate animals—and especially chickens—may make a significant contribution to the onsite burden of feces. In fact, recent evidence has suggested onsite fecal contribution from animals may be more than feces from humans, including in urban areas [300]. Non-human fecal contamination by domestic or wild animals can contribute to detection of fecal indicators and may indicate presence of zoonotic enteric pathogens [5]. Consistent with a cross sectional study in Bangladesh, chickens were associated with higher *E. coli* counts in soil compared to other animals [97]. The ubiquitous fecal contamination observed in this and other studies [97,100,217] in low-income settings may limit the ability for WASH interventions to consistently reduce environmental fecal contamination [32]. Future iterations of sanitary surveys would benefit from including the presence of animals or animal feces as inputs.

After feces is introduced to the environment, the persistence of enteric pathogens is dependent on time, temperature, soil moisture content, and exposure to UV radiation from sunlight among other factors [192]. Consistent with other studies, we found concentrations of

E. coli in soil to be associated with the sun exposure status of a sample and whether the sample was visibly wet [97,98]. Despite sampling during the dry season, nearly two-thirds of soil samples were visibly wet, and we observed standing water at almost half of compounds. Unsurprisingly, we detected *E. coli* most frequently from locations where soil was most frequently visibly wet, the areas where water-based activities such as dishwashing and clothes washing were performed [98].

In sanitation assessments latrine entrances are typically assumed to be directly impacted by the intervention. However, among the nine intra-compound locations we tested *E. coli* at the latrine entrance was the third most prevalent and sixth highest in concentration. The heterogeneity of *E. coli* concentrations among intra-compound locations emphasizes the importance of spatial standardization for soil sampling. Soil samples should be collected from locations where similar activities are performed across sites. Our results suggest that sites such as a child's most recent play area or where a child most recently spent time [52] may not be sufficiently standardized for soil sampling in this and similar contexts.

Swabs of common compound surfaces yielded infrequent detection of fecal contamination across surfaces in this context. We most often detected *E. coli* on plastic chairs, which we suspect is a result of swabbing the horizontal seat of the plastic chair which may collect dirt and debris. All entrance swab surfaces were vertical, while kitchen related surfaces are typically cleaned regularly. A similar study in Tanzania found vertical latrine wall surfaces had the lowest *E. coli* counts compared to other common household surfaces [98]. We did not account for how recently each surface was cleaned, which may have been heterogenous and we did not specify the type of child's play toy or food preparation surface for swab sampling. These factors may explain limited detection of *E. coli* on surfaces.

Exclusively swabbing horizontal surfaces such as floors [270], or identical sentinel objects such as a child's play toy, may be better approaches to standardize swab surfaces among households [219,271,301].

E. coli in soil is an imperfect indicator of sanitation-related fecal contamination in this context and the *E. coli* we detected may not have come from human sources, as supported by our observed associations between chicken presence and *E. coli* in soil. Previous work has suggested *E. coli* may be indigenous to soils in the tropics [302,303]. Soil-borne *E. coli* can grow and replicate when incubated at 30-37ºC and can persist longer than one month when temperatures exceed 25ºC, which is common year-round in Maputo [55]. Furthermore, not all *E. coli* are pathogenic and *E. coli* do not serve as an adequate indicator for enteric pathogens in many settings [100,217,304]. Further molecular analyses of these samples will be useful to understand whether and to what extent enteric pathogens are detected in soils from these sites.

Our study has several important limitations. The sample size of 80 compounds limited the number of covariates included in models and statistical power, including multivariable assessment of variables (such as the presence of chicken or ducks) that were infrequently observed. Additionally, we did not collect data to differentiate between compounds with penned animals and free-roaming animals, which may have impacted local environmental fecal contamination. The LSSI did not include disposal of children's feces, which, if improperly disposed of, may be spread fecal contamination into the environment [146]. The LSSI included observed human feces in and around the latrine, but open defecation rates are difficult to capture in a cross-sectional study and may vary among households in a compound [218].The pre-selection of compounds enrolled in the MapSan trial was purposive;

thus our conclusions may not be generalizable to all compounds in low-income areas of Maputo, or broader contexts. The range of the LSSI in the compounds we sampled did not include many compounds with LSSI values close to 0 and 1; a larger sample size may be useful in future research to capture compounds at the extremes. The absence of association between LSSI quartiles and continuous *E. coli* counts may have been due to a small sample size or may suggest a non-linear relationship and could be an area of future research. LSSI weights developed from surveys of local sanitation experts may have been subjective and may not have best associated with localized fecal contamination. Substantial heterogeneity existed between sample location and sample type despite our intention to select comparable sites for soils and swab samples between compounds. Other statistical approaches may be more useful to optimize the LSSI. For example, future research could use decision tree analysis to determine which variables have the greatest impact on fecal contamination.

In low-income, pathogen- and fecal contamination-rich, urban settings where sanitary conditions are poor, our study suggests better sanitary conditions measured via a sanitary survey may be associated with lower measures of environmental fecal contamination relative to poorer scores, though the absolute difference in contamination between poor and better sanitary conditions is minor and the association we found was borderline significant. There was no significant difference in the complete LSSI's association with concentrations and detection of *E. coli* in soil compared with the unweighted LSSI alternative, suggesting a need for improved variable selection and weights. Further research should explore the inclusion of animals as sanitary survey inputs and how to optimize sanitary survey weighting schemes. The LSSI provides a helpful first iteration of a proxy

for environmental fecal contamination in low-income settings where analysis of environmental samples is not feasible.

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